Haemolytic uremic syndrome: from bedside to bench to bedside

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HAEMOLYTIC UREMIC SYNDROME: FROM BEDSIDE TO BENCH TO BEDSIDE

HABILITATIONSSCHRIFT

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Zusammenfassung

Das hämolytisch-urämische Syndrom (HUS) ist eine Krankheit, die sich bei den meisten Patienten im Kindes- und Jugendalter manifestiert. Das HUS zeichnet sich durch die Trias hämolytische Anämie, Thrombozytopenie und akute Niereninsuffizienz aus. Histologisch und pathopysiologisch liegt der Erkrankung eine thrombotische Mikroangiopathie (TMA) zu Grunde. Hierbei kommt es zur Schädigung der Endothelzellen und zur Bildung thrombotischer Verschlüsse in den kleinen Gefässen, insbesondere in den Nieren. Das HUS ist jedoch eine Systemerkrankung; daher treten schwerwiegende Komplikationen auch in anderen Organen auf wie z.B. Gehirn, Augen, Herz, Pankreas.

Historisch wurde das HUS in Durchfall-positives HUS (sog. „typisches“ HUS) und Durchfall-negatives HUS (sog. „atypisches“ HUS) eingeteilt. Im ersten Fall wird das HUS durch Infektionen mit Shigatoxin-produzierenden Escherichia coli (STEC) verursacht. Das „atypische“ HUS, das die rezidivierende HUS Formen beinhaltet, ist STEC negativ. Im Laufe der letzten Jahrzehnte wurde die HUS Klassifikation mit neu gewonnenen pathophysiologischen Erkenntnissen fortlaufend geändert und an die Vielfalt der HUS-Ursachen angepasst. Insbesondere die Rolle des Komplementsystems in diesem Krankheitsbild prägt die aktuelle HUS-Klassifikation: Infekt-assoziiertes HUS (u.a. STEC, Pneumokokken, andere Infektionen), atypisches HUS (genetisch determinierte Dysregulation des alternativen Komplementsystems), HUS bei Cobalamin (C) Defekt und HUS assoziiert mit anderen Systemerkrankungen (z.B. autoimmun Erkrankungen, Organtransplantation, Tumoren).

Auf Grund der Seltenheit und der Vielfältigkeit des HUS ist die Durchführung von doppelblind kontrollierten klinischen Studien kaum möglich. Es gibt einige Langzeitstudien betreffend Mortalität und Nierenfunktion. Aber genaue Daten zum neurologischen und kognitiven Outcome sowie der Lebensqualität von Kindern nach HUS fehlen. Daher gewinnen klinische Outcome-Studien und Fallserien bei diesem seltenen Krankheitsbild einen besonderen Stellenwert, um experimentelle Erkenntnisse besser verstehen und erweitern zu können.

Mit dieser Arbeit möchte ich deshalb anhand von Outcome-Studien einen klinischen Ansatz zur Erfassung sowie zur Optimierung der Lebensqualität betroffener Kinder und deren Eltern nach lebensbedrohlichem HUS vorstellen. Zudem soll diese Arbeit zeigen, wie in den letzten Jahren auf dem Gebiet der thrombotischen Mikroangiopathie neue Perspektiven für das Verständnis der Pathophysiologie und für neue Therapieoptionen sowohl für das HUS, aber auch für andere Glomerulopathien eröffnet wurden. Obwohl das HUS bisher vor allem als pädiatrische Entität verstanden wurde, konnten in den letzten Jahrzenten neue Erkenntnisse

insbesondere im Bereich des Komplementsystems für die Diagnose und Therapie von Krankheiten gewonnen werden, die vorwiegend in der Adult-Nephrologie auftreten wie z.B. membranoproliferative Glomerulonephritis und weitere Glomerulopathien (assoziiert mit Schwangerschaften, Medikamenten, Systemerkrankungen u.a.). Zudem können renale Langzeitkomplikationen (u.a. Proteinurie, Hypertonie, Niereninsuffizienz) auch erst nach einem längeren „freien“ Intervall im erwachsenen Alter auftreten. Deshalb ist eine formelle Transition und kontinuierliche nephrologische Betreuung dieser Patienten erforderlich ist.

Mit den klinischen Studien bei Kindern mit HUS (original articles 1-3) haben wir im ersten Teil sowohl die akuten neurologischen Komplikationen, als auch den neurologischen und kognitiven Langzeitverlauf erfasst. Im zweiten Teil wurden die Lebensqualität und die psychologische Anpassung der Patienten analysiert. Im dritten Teil wurde die Lebensqualität und psychische Verfassung der Eltern von Kindern nach durchlebtem HUS untersucht.

Die therapeutisch-klinische Studie (original article 4) beschreibt anhand einer klinischen Fallserie die Beteiligung des alternativen Komplementsystems bei der membranoproliferativen Glomerulonephritis und C3-Glomerulopathie. Wie beim HUS können auch bei diesen Krankheiten Genmutationen von Komplementfaktoren gefunden werden, welche zur Dysregulation des alternativen Komplementsystems führen. Entsprechend kann wie beim HUS eine komplementregulierende Therapie erfolgreich eingesetzt werden.

Summary

The haemolytic uremic syndrome (HUS) is a disease syndrome presenting mainly in childhood and adolescence. It is characterized by the triad of haemolytic anemia, thrombocytopenia and acute renal failure. Histology and pathophysiology demonstrate a thrombotic microangiopathy (TMA) which leads to damage of endothelial cells and to the formation of thrombotic occlusions in small vessels, in particular in the kidneys. HUS, however, is a systemic condition with complications occurring in other organs including e.g. brain, heart, eyes and pancreas.

Historically, HUS has been classified into diarrhea-positive HUS (so- called “typical” HUS) and diarrhea-negative HUS (so-called “atypical” HUS). In the former, HUS is predominantly caused by infections with Shiga toxin-producing Escherichia coli (STEC), while “atypical” HUS, with recurrent HUS episodes, is STEC negative. Over the last decades, the HUS classification was updated continuously, given new pathophysiological findings, and therefore has been adapted to the variety of HUS causes. Particularly, the role of complement system in this disease underpins the current HUS classification: infection-associated HUS (including STEC, pneumococcal, other infections), atypical HUS (genetically determined dysregulation of the

alternative complement system), Cobalamin (C) defects and HUS associated with other systemic diseases (e.g. autoimmune diseases, organ transplantation, malignancies)

Due to the rarity, the variability and severity of this disease, and for ethical-moral reasons, the performance of double-blind, controlled studies is hardly possible. Consequently, well-validated long-term data on the neurological, cognitive outcome and quality of life of children after HUS are scarce. Knowledge of clinical outcomes in studies and case series are of particular importance to inform this rare clinical picture, in order to reach a better understanding and to expand research knowledge.

Based on the new translational perspectives described in recent years in the field of thrombotic microangiopathy leading to a better understanding also of other glomerulopathies, new therapeutic options, will be discussed. Although HUS has thus far been a primarily pediatric entity, in recent decades new insights have been gained, particularly in the field of the complement system, in forming diagnosis and treatment of diseases that predominantly occur in adult nephrology including membranoproliferative glomerulonephritis and other glomerulopathies (e.g associated to pregnancy, to drugs, to systemic diseases). In addition, renal long-term complications after HUS (including. proteinuria, hypertension, renal insufficiency) can manifest at adult age, therefore a nephrology care across the life-course of these patients is required.

Here, I present a clinical approach for detection and optimization of the quality of life in affected children and their parents after life-threatening HUS. In the first part of the clinical studies (original article 1-3), both the neurological complications as well as the neurological and cognitive long-term course in children after HUS were recorded. In this context, in a second part, the quality of life and psychological adjustment in children after HUS by applying standardized questionnaires, were assessed. Simultaneously, the quality of life and mental health of the parents of children who survived HUS, were analyzed.
A therapeutic-clinical study (original article 4) describes the involvement of the alternative complement system in membranoproliferative glomerulonephritis and C3 glomerulopathy based on the experience of a case series. As in HUS, also in these glomerulopathies mutations of complement factors leading to dysregulation of the alternative complement system are involved. Therefore, as in HUS a complement regulation therapy may be possible also for other glomerulopathies,

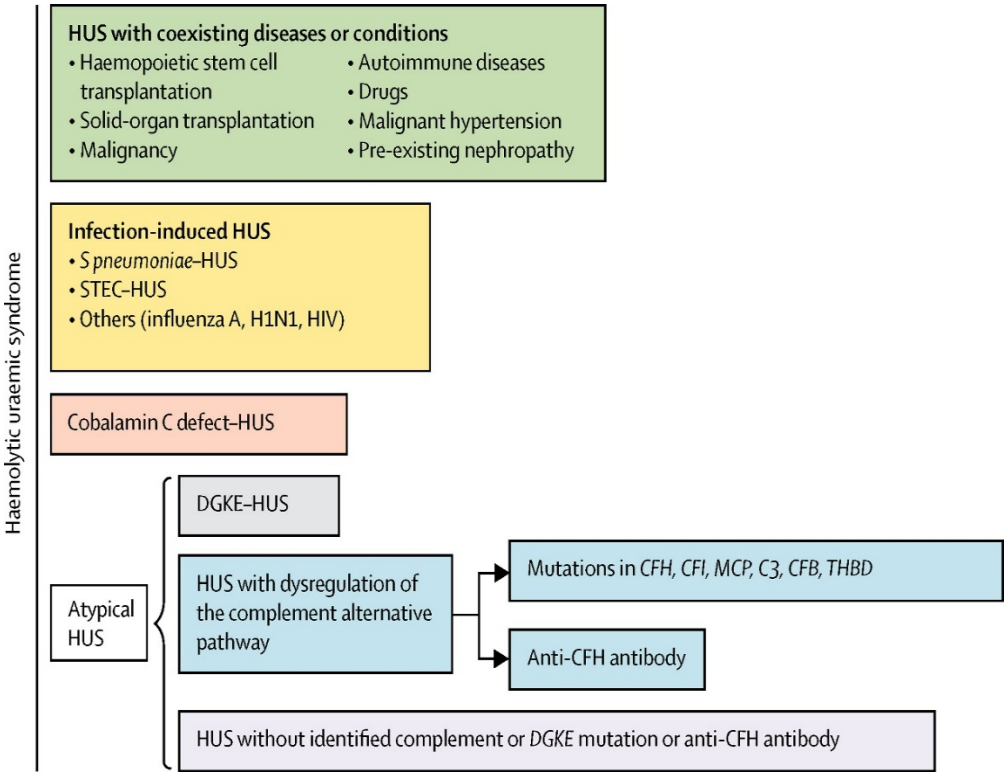
Introduction

Haemolytic uremic syndrome: from Zurich to worldwide renown

In 1924, Eli Moschcowitz, an American internist of Hungarian origin, was the first to describe a new clinico-pathological entity obeserved in the autopsy of a young woman who died after developing acute febrile haemolytic anemia with petechiae and neurological signs (mild left hemiparesis and facial paralysis). He described the unique clinical and post mortem histological features and named the disease *thrombotic thrombocytopenic purpura (TTP)* (Moschcowitz E 1924). Three decades later, in 1952, William St. C. Symmers, a pathologist at the Charing Cross Hospital in London, coined the term *thrombotic microangiopathy (TMA)* to indicate the location and the most striking characteristic features of the histological lesions: widely disseminated thrombosis of the smallest-caliber blood vessels, with endothelial hyperplasia, important dilatation of the affected vessels, an no inflammatory reaction. Close collaboration with Symmers led Conrad Gasser, a Swiss paediatric haematologist at the Kinderspital Zurich, to describe a new entity 'haemolytic uraemic syndromes' (HUSs) in 1955. He reported five children with microangiopathic haemolytic anaemia, thrombocytopenia, and acute renal failure. Two each of the patients had prodromal diarrhea and pleuropneumonia; one patient had no prodromes (Gasser C 1955). The plural form of HUS ('Syndrome' in German) was deliberately used in anticipation of the epidemiological, clinical, and pathophysiological heterogeneity of the disease. Contrary to TTP, severe renal involvement is a hallmark of HUS.

Over the last 10 years many advances have been made in the field of HUS. New pathogenic mechanisms have been identified (e.g genetic or acquired dysregulation of the alternative complement pathway) and innovative effective treatment has become available. The classification of HUS has been adapted several times in the last years. In 1998 the current classification was divided into the typical diarrhea associated HUS due to infection from shiga toxin-producing Escherichia coli (STEC), and atypical HUS, with the latter having a more severe, and in some cases relapsing course. The current classification of HUS considers various forms of HUS which can occur as complication of, or be precipitated by, various diseases and conditions as described in *figure 1.*

Classification of various forms of haemolytic uremic syndrome



STEC=Shigatoxin-producing Escherichia coli

DGKE=diglycerolkinaseε

CFH=complement factor H

CFI=complement factor I

MCP=membane-cofactor protein

C3=complement C3

CFB=complement factor B

THBD=thrombomodulin

Figure 1: From Fakhouri F; Lancet 2017

The incidence of HUS in central Europe is estimated to be 1 to 1.5 patients/ 100 000 children and adolescents under 16 years. In Switzerland approximately 20 HUS-cases occur every year as reported by the Swiss Paediatric Surveillance Unit in a prospective national study (Schifferli A 2010). In this registry, typical, diarrhea- associated HUS occurred in 90% of the children and STEC was isolated in 60% of the tested blood samples. The mortality rate was 5.3% with the highest mortality in pneumococcal-associated HUS. The severity of thrombocytopenia and the presence of central nervous system involvement significantly correlated with mortality.

Children aged one to five years are most by affected, however this disease can occur at any age, with a peak in the second and third year of life. In Switzerland HUS is the most common cause of acute kidney injury (AKI) requiring renal replacement treatment in childhood. Mortality is low, but the rate of long-term renal and extrarenal complications, i.e., proteinuria, hypertension and chronic kidney disease is significant.

Classification of thrombotic microangiopathy (TMA)

TMA reflects the unifying histopathological description, encompassing both TTP and HUS, clinically characterized by thrombocytopenia, microangiopathic haemolytic anemia and organ injury (Moake JL 2002). Acute kidney injury and neurological symptoms are common and prominent feature because of the vulnerability of the glomerular and brain circulations to endothelial damage and occlusion. TMA is associated with significant mortality and morbidity, including end stage renal disease (ESRD); however, prompt initiation of supportive and specific management can alter outcome (George JN 2014).

The classification of TMAs is challenging and constantly evolving. Historical diagnostic classifications were based on clinical findings: TTP when neurologic involvement predominated and HUS when kidney disease was predominant. Therefore, until the late nineties, confusion between TTP and HUS persisted (some authors referred to the syndrome as HUS/TTP). Classifications have evolved as understanding of the molecular basis of disease has improved: TTP is defined by a severe congenital or acquired ADAMTS13 deficiency, Diarrhea-associated HUS is defined by the presence of Shiga toxin-producing E. coli (STEC-HUS), and atypical HUS (aHUS) is used for other causes of TMA. The discovery of the role of complement in patients with aHUS has subsequently led to the term *complement-mediated TMA* (George JN 2014; Nester C 2015). Inconsistencies in the historical and current literature over nomenclature make interpretation difficult: aHUS may refer specifically to complement-mediated TMA, or be more generally applied to any TMA that is not TTP or STEC-HUS. In the recent literature (Brocklebank V et al 2018), the *term complement-mediated aHUS* is used when the cause is defined as such, and the term aHUS is used when the cause is ill-defined. Current classifications describe primary TMAs, either acquired or congenital (e.g. complement mutations, ADAMTS 13 mutations), secondary TMAs, and infection-associated TMAs (*figure 2*).

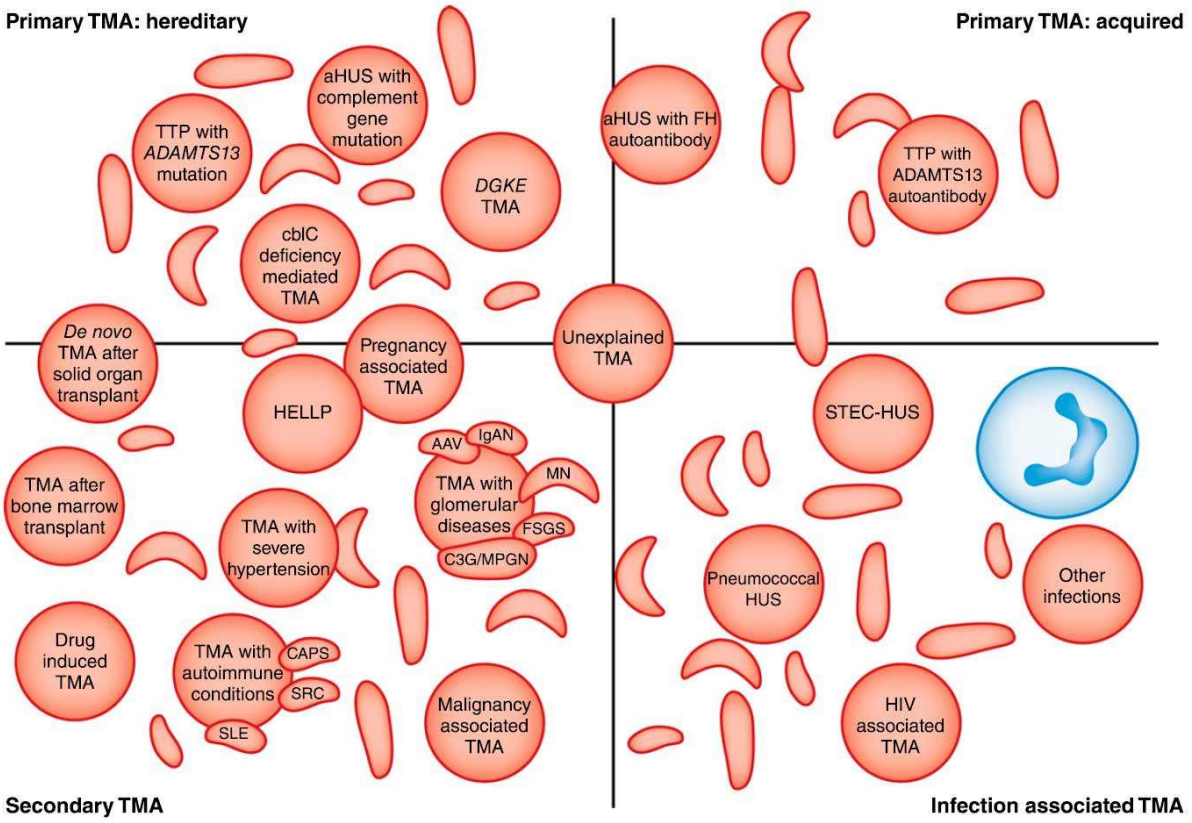


Figure 2: From Brocklebank V; CJASN Feb 2018

Current classification define primary TMAs as hereditary or acquired, or autoantibodies to complement H. Secondary TMAs occur in a spectrum of conditions and in many cases the pathogenic mechanism are multifactorial or unknown. TMAs may be also associated with various infections as STEC and pneumococcal HUS. In other infections, the processes are not defined and in some cases the infection may trigger manifestation of primary TMA.

These terms are however too simplistic as they do not account for the increasing recognition that patients with an underlying complement risk factor often require a secondary trigger for TMA to manifest. Mutations in the genes of complement factor proteins have been found in about 60% of patients with the atypical form of HUS (aHUS).

The role of the innate Immune system and the complement cascade

Humans are permanently exposed to micro-organisms and could not exist as species without a highly effective mechanism of host defense. The innate immune system constitutes the first-line barrier to prevent microbial invasion. Its components are inherited from parent to child and are directed against molecules expressed only by micro-organisms. The term „innate“ immunity refers to immune responses that are present from birth. This is in contrast to the “adaptive” immune system which is based on up-regulation, adaption and repeated boostering as a result of recurrent and/or constant exposures to micro-organism. Considering the temporal aspect, there is a difference between the two immune systems: the response time of the „innate“ immune system the pathogens is 20 to 30 minutes, whereas the development of a specific “adaptive” immune response mediated by T-cells and antibodies takes days to

weeks. Therefore, the innate immune system protects the host from the time of microbe exposure to elaboration of the adaptive responses.

The complement system constitutes an essential part of the innate immune system. It guards the host's intravascular space by opsonizing and lysing bacteria. In addition, it promotes the local acute inflammatory response, which in turn instructs and influences the adaptive immune response.

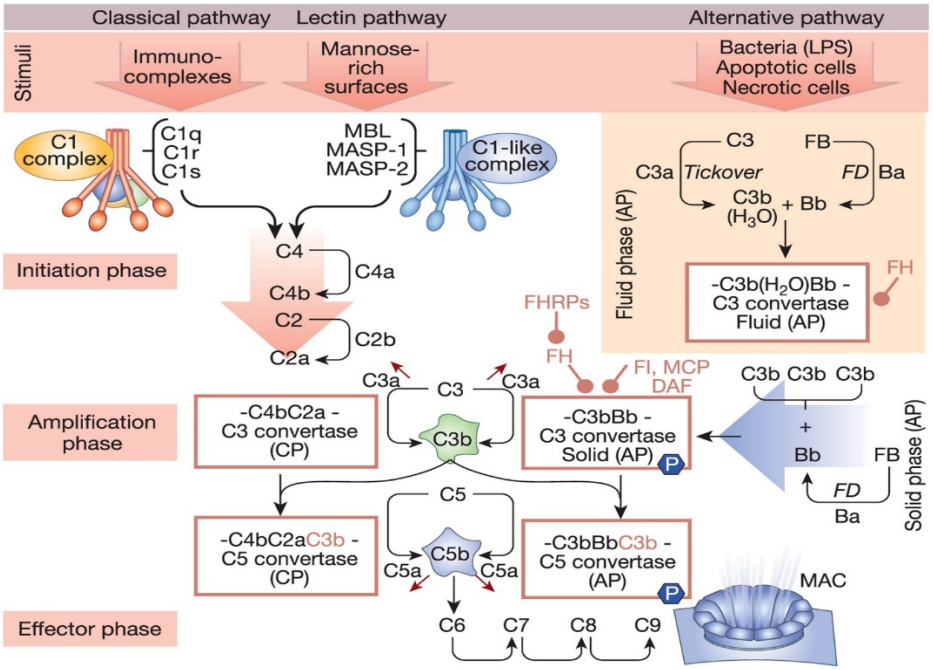
The complement system is a proteolytic cascade, comprising more than 30 proteins, where serine proteases activate each other in a strictly ordered manner. The complement components exist in soluble form called the *fluid phase*, or are expressed on the cell membrane, the *solid phase* (Angioi A 2015). The plasma proteins interact via three major cascades: the classical, the lectin and the alternative pathways. Each of the 3 complement pathways is triggered by a distinct set of conditions (e.g. antibodies binding to antigens, sugar molecules on the surface of pathogens or damaged host cells, respectively). All three pathways generate a proinflammatory environment, with the common goal of modifying the target membrane by deposition of C3 activation products (opsonization) and then engaging a common terminal sequence or pathway called the « membrane attack complex » (MAC) which leads to membrane perturbation and cell lysis. The successive phases include attachment (initiation phase after "stimuli"), activation and amplification (amplification phase/convertase formation), and membrane attack (effector phase/membrane perturbation) (Angioi A 2016). Rigorous active control mechanisms are required to prevent damage to self (*figure 3).*

The classical pathway is most commonly triggered by antibodies binding to antigens. This pathway is activated by interaction between C1q and immune complexes (immunoglobulin G or immunoglobulin M). The serine proteases C1r and C1s are then activated by binding to the C1q-immunoglobulin complex. This, triggers an autoactivated cleavage process leading to amplification of complement components (C4, C4a, C4b, C2, C2a, C2b). The C4b fragment combines with the lipid bilayer of the target cell and C2a to form the C3 convertase of the classical pathway, C4b2a.

The lectin pathway, also called the mannan-or mannose-binding pathway, is similar to the classical pathway, and generates an identical C3 convertase (C4b2a). Lectins are proteins that bind to sugars. Mannose-binding-lectin (MBL) is part of the acute-phase response. MBL binds to MBL-associated serine proteases (MASP-1, MASP2) that cleave intact C4 and C2 to generate the C4b2a convertase).

The alternative pathway is an ancient pathway of innate immunity preceding adaptive immunity. Thus, the alternative pathway does not require antibodies or prior contact with microbes to function and become activated. It serves as an independent immune system, capable of recognizing and destroying infectious elements. In contrast to the classical and

lectin pathways, the alternative pathway (AP) is capable of autoactivation. Indeed, C3 is constantly autoactivated (so-called « C3 tickover ») at a low level. This process is rapidly amplified in the presence of microbes, damage to host cell, or when complement regulatory proteins are deficient. Deposition of C3b on target cells can be efficiently amplified by the alternative pathway's **feedback loop** *(figure 3 and figure 4).*



AP = alternative pathway

CP = classical pathway

Figure 3: From Angioi A; Kidney Int 2016

In summary, the alternative system is a complex-interacting system which is engaged when activated C3 binds factor B. Bound factor B undergoes proteolytic cleavage mediated by another serine protease, factor D (FD), to produce the fragments Bb and Ba which are released into the surrounding milieu (Forneis F 2010). The alternative pathway C3 convertase, C3bBb, is then stabilized by properdin (P), creating the complex C3bBbP. As the convertase cleaves more C3 to C3b, an amplification loop is set in motion, resulting in the deposition of large amounts of C3b on the target cells *(figure 3).*

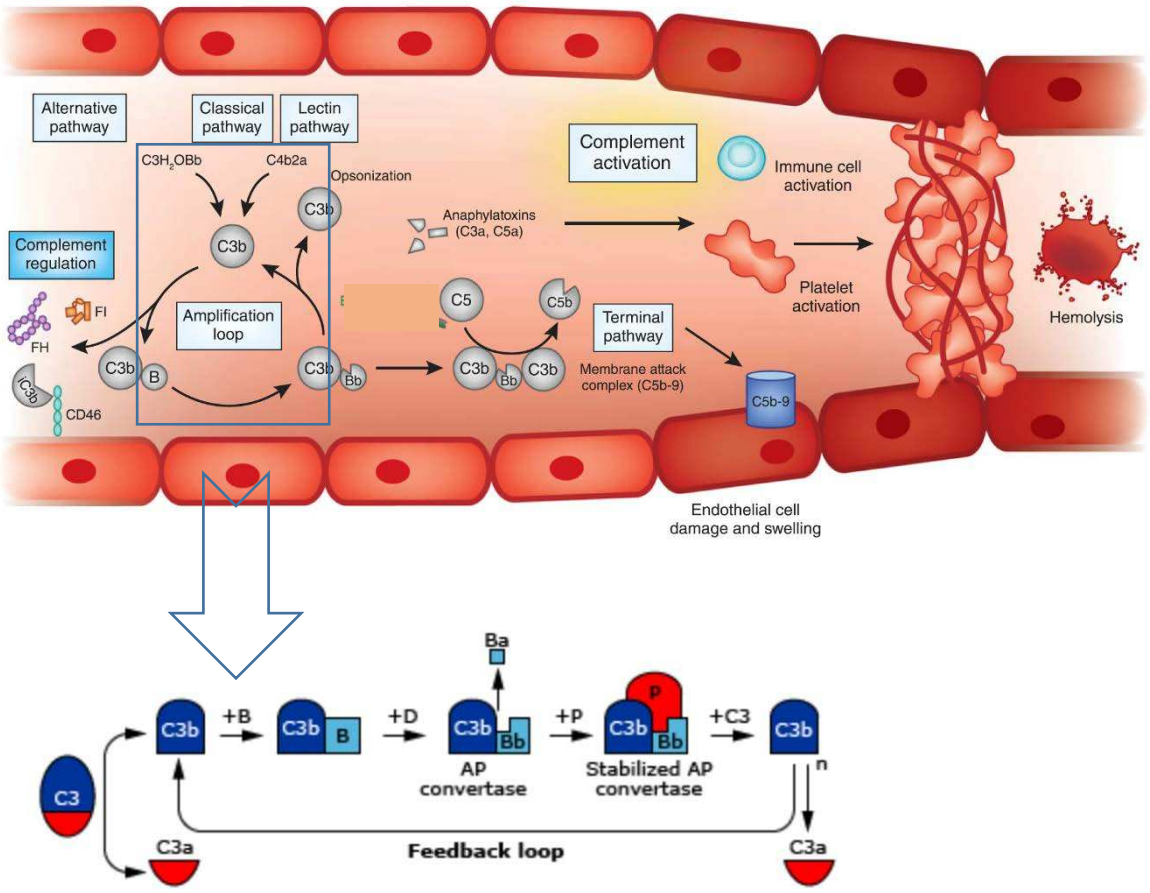
C3b, deposited by the classical or lectin pathway, can serve as a nidus for amplification by the alternative pathway. In many clinical situations, the initial deposition of C3b is mediated by the classical or lectin pathways. The C3b is then amplified many-fold through the feedback loop of the alternative pathway.

Finally, the alternative pathway also engages the MAC which is then assembled as in the classical pathway.

During complement activation, host cells are protected from damage by plasma and membrane regulatory proteins (complement factor I (FI), complement factor H (FH), MCP, decay-acceleration factor (DAF), complement factor H related proteins (FHRPs) *(figure 3).* Therefore, it is essential to understand the role of complement in the normal physiological setting in maintaining homeostasis and keeping the organism intact and healthy. Consequently, inappropriate timing, dysregulation, misdirected delivery of complement effector products or any effect that disturbs the delicate balance of this complex network of interacting proteins can result in pathology and in numerous diseases, such as ischemia reperfusion injury, Systemic Lupus Erythematodes (SLE), and others (Zipfel PF 2009).

In HUS, platelet dysfunction and damage of the endothelial cell lining of kidney blood vessels are primary events that result in microvascular lesions and in the formation of microthrombi occluding the small blood vessels in the kidney *(figure 4).*

Complement pathway, its regulators and formation of microthrombi in TMA



Alternative Pathway

Figure 4: Adapted from Brocklebank V CJASN Feb 2018 and Liszewski MK, Immunology Scope Monograph 1992

Biochemical and genetic abnormalities in HUS and TMA

The defining laboratory features of HUS include the triad of: 1) microangiopathic haemolytic anemia, identified by erythrocyte fragmentation on peripheral blood film microscopy, caused by a turbulent flow in the microcirculation due to partial occlusion by platelet aggregates, elevated lactate dehydrogenase as a result of cell lysis and tissue ischemia, reduced haptoglobin and a negative direct antiglobulin (Coombs) test (except in pneumococcal HUS); 2) thrombocytopenia resulting from platelet aggregation and consumption; 3) acute kidney injury. The renal microcirculation has a high shear stress and turbulent flow making it vulnerable to thrombotic microangiopathy (Bettoni S 2017). The kidneys therefore are usually the first organ affected by complications in HUS. Once TMA is demonstrated by the above mentioned routine biochemical and haematological analysis, further investigations are aimed at determining the underlying disease cause. In this setting, genetic investigation of complement disorders may be crucial to make an accurate diagnosis (*Table 1*).

As described above, the complement system is tightly regulated by the circulating plasma “fluid phase regulator proteins”, mainly produced in the liver, including e.g. complement Factor H (CFH) and factor I (CFI), and cell surface “membrane-bound regulator proteins” such as membrane cofactor protein (CD46).

Defects in these regulators or in the alternative pathway components, associated with either increased or impaired activity, can lead to complement dysregulation, activation of the terminal complement pathway, generation of the anaphylatoxin C5a and the membrane attack complex (C5b-9), finally resulting in complement-mediated aHUS.

In complement–mediated aHUS, dysregulated complement activation occurs primarily on the endothelial cell surface, and although abnormal serum levels of complement components, such as low C3 may be observed, normal levels do not exclude complement-mediated disease (Goodship Th 2017; Kavanagh D 2013). Molecular evidence that *CFH* mutations are associated with aHUS was first described in 1998 (Warwicker P et al 1998). Since then several studies have shown numerous heterozygous pathogenic activating mutations in the genes encoding the alternative pathway components C3 and factor B, and loss-of-function mutations in the genes encoding the regulators FH (including CFH/CFHR -- complement factor H-related -- fusions) and FI, and CD46 (Kavanagh D 2013). These genetic mutations are not causative, but instead predispose to HUS, with incomplete penetrance. The penetrance of disease is age-related and has been reported to be as high as 64% by the age of 70 years for individuals carrying a single genetic mutation. This observation highlights that additional disease risk modifiers are essential. Approximately 3% of patients have more than one mutation in the complement system genes, with increased penetrance per additional mutation. Haplotypes (particular combinations of single nucleotide polymorphisms) in CFH and CD46 also modify

penetrance (Fakhouri F 2017; Fremeaux-Bacchi 2013). Atypical HUS is also often associated with high risk of disease recurrence after renal transplantation. However, a combination of certain heterozygous mutations (e.g. MCP/CFI) might have a beneficial impact on the course after renal transplantation, predicting a lower risk of aHUS recurrence in the renal graft (Pabst WL et al 2013). Together, these observations do not answer the question why some individuals never develop the disease or do so only in later life. Such variability might be explained by the need for a second hit, e.g., an environmental trigger such as pregnancy or infection which may unmask a latent complement defect. Complement activation is a common factor in many of these triggering events. Atypical HUS associated with autoantibodies against FH was first reported in 2005 (Dragon-Durey MA 2005). Moreover, functional analyses have demonstrated a disruption of complement regulation by multiple mechanisms (Blanc C 2012). A strong association of CFH-antibodies to homozygous deletion of CFHR3 and CFHR1, enconding the proteins FHR3 and FHR1 is described, although the mechanism is not understood; CFHR3/1 deletion is a common polymorphism, but is not present in all individuals who develop CFH autoantibodies (Brocklebank V 2017). This form of aHUS predominantly presents in childhood, frequently with a gastrointestinal prodrome. Autoantibodies against FI have also been reported, but they are rare and their functional relevance remains to be established (Kavanagh D 2012). Also genetic variants in thrombomodulin (THBD) have been reported in association with aHUS (Delvaeye M 2009). In pneumococcal HUS, involvement of a vitronectin-binding adhesin of serotype 3 pneumococci binding to CFH was recently suggested, leading to activation of the complement cascade and HUS (Kohler S 2015).

Recently, interaction between von Willebrand factor (VWF) and complement has been described (Bettoni S 2017). ADAMTS 13 is a metalloproteinase cleaving ultralarge (UL-) VWF multimers into numerous small fragments on the surface of endothelial cells. In congenital TTP (ADAMTS 13 deficiency) or in acquired TTP (anti-ADAMTS 13 antibodies), VWF multimers cannot be cleaved because of ADAMTS 13 deficiency. Therefore, in the absence of ADAMTS 13 the endothelium is exposed to abundant deposition of unfractionated VWF multimers resulting in increased thrombogenicity. Consequently, activated complement system is leading to microvascular injury of endothelial cells and to TMA.

Recessive mutations in diacylglycerol kinase (DGKE) may result in protein kinase C activation, leading to upregulation of VWF and tissue factor resulting in a prothrombotic state and inducing TMA (Lemaire M 2013). The outcomes of TTP and HUS treatment strategies have been inconsistent in DGKE-induced HUS (Brocklebank V 2017).

• CFH, anti-CFH-Ab and CFHR 1-5, • CFI • CFB • C3 • Thrombomodulin • MCP/CD46 • DGKE • ADAMTS 13 and Anti-ADAMTS 13 antibody (Ab) • Cobalamin-C defect	**Genetic complement investigation in TMA (HUS/TTP)**

Table 1

Clinical features and complications in HUS and TMA

The clinical presentation of HUS is common also for TMA, reflecting haemolysis and ischemic organ dysfunction, and depends on the underlying disease etiology: acute kidney injury is therefore a common manifestation in TMA although rarely a severe feature of TTP. Extrarenal manifestations do occur in aHUS and STEC-HUS, however it is not known whether they are a consequence of the TMA, a direct effect of complement activation or shiga toxin, or complications of AKI, such as severe hypertension and uremia.
Extrarenal complications have been reported in 19-26% of the HUS cases (Schifferli A 2010; Mattheis J 2016). The clinical presentation varies from mild disease to fulminant course, including death.

Extrarenal complications:

- Neurological involvement: e.g. seizures, altered consciousness, cerebral haemorrhage, encephalopathy
- Gastrointestinal involvement: e.g. diarrhea, vomiting, abdominal pain, intussusception, bowel obstruction, sclerosing cholangitis, hepatocellular cholestasis, pancreatitis, diabetes mellitus
- Cardio-vascular involvement: cardiomyopathy, cerebral arterial thrombosis/stenosis, extracerebral artery stenosis, myocardial infarction, digital gangrene/ skin necrosis
- Ocular involvement: isolated intraretinal haemorrhages, Purtscher-like retinopathy with retinal ischaemia, visual impairment
- Pulmonary involvement

New therapeutic options

The use of eculizumab in complement-mediated HUS based on pathophysiology and in - selected cases of - STEC-HUS, has permitted more rapid and specific treatment in recent years, especially in cases with severe neurological complications.

Eculizumab is therefore considered first-line treatment if a complement-mediated aHUS is suspected and TTP has been excluded. In the pre-eculizumab era, one option for individuals with a defect in a complement protein predominantly synthesized in the liver (CFH, CFI CFB and C3) who had developed ESRD was combined liver and kidney transplantation (Saland J 2014). However, this procedure was associated with significant perioperative complications and mortality. Therefore, long-term treatment with parenteral eculizumab in complement-mediated HUS has revolutionized the therapeutic approach and improved the outcome. In addition, knowledge gained from treatment of complement-mediated HUS, has led to the use of this complement- modifying/inhibiting therapy for other causes of TMAs.

Elucidation of the central role of primary complement defects in the pathogenesis of aHUS provided the mechanistic rationale for treating complement-mediated aHUS with complement –inhibiting therapy. Eculizumab is a recombinant humanized antibody that functionally blocks C5, and seminal trials published in 2013 demonstrated its excellent efficacy (Legendre CM 2013). Although these were single-arm studies rather than randomized, controlled trials, the historically poor outcomes of aHUS justified such study designs. In prospective trials, complete TMA response was achieved in approximately 65% of patients after 26 weeks of eculizumab therapy in both adults (Legendre CM, 2013) and children (Greenbaum LA 2016)

With increasing clinical use however, evidence is emerging of nonresponse to eculizumab in aHUS. A recent clinical trial of Greenbaum et al (Greenbaum LA 2016) highlighted that, those with a rare genetic variant in the complement system or autoantibodies to complement factor H, all had an improvement in estimated glomerular filtration rate (eGFR), whereas 27% of individuals without an identified complement abnormality failed to show any improvement. It is not clear whether this lack of response reflects a non-reversible organ damage or true nonresponse.

The role of complement activation as a second- hit amplifying endothelial cell damage in TMA is suggested in STEC and pneumococcal- induced HUS, as well as in several secondary forms of TMA.

Typical, STEC-HUS is caused by verotoxin (Shiga toxin (Stx)) released by bacteria (e.g. E. coli, Shigella), which can be detected in stool samples by PCR. The toxin is absorbed by the gut, reaches the circulation, and leads to alteration of the endothelium of the microcirculation, thereby triggering the alternative complement pathway, resulting in the formation of microthrombi in end-organs *(figure 5).*

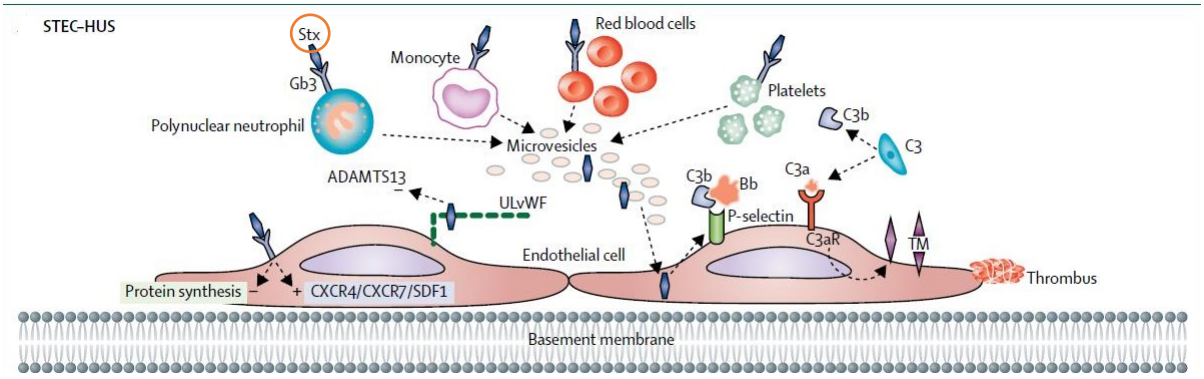


Figure 5: From Fakhouri F, Lancet 2017. In STEC-HUS, Stx enters the endothelial cell via Globotriasylceramide (Gb3)-dependent and Gb3-independent pathways, and exerts its cytotoxic effect via protein synthesis inhibition and enhancement of the CXCR4/CXCR7/SDF1 pathway. CXCR4/CXCR7 are 2 G-protein-coupled receptors and there are shared cognate chemokine ligand (SDFl=stroma cell derived factor1) as key target of Stx-induced mRNA expression. Stx also induces the translocation of P-selectin (a cell adhesion receptor) to the endothelial cell surface, favouring the assembly of alternative C3 convertase, the release of C3a and thrombomodulin (TM).

Renewed interest in HUS arose during the severe outbreak in Germany in 2011 (Kielstein JT 2011). In a retrospective registry analysis the various therapies applied during outbreak were compared (supportive care, plasmapheresis and eculizumab). Despite frequent renal impairment, advanced neurological disorders and severe respiratory failure, short-term outcome was better than expected when compared with previous reports. However, given the retrospective non-randomized nature of the intervention and analysis, no evidence-based indication for eculizumab in STEC could be established. Latter was also confirmed recently by Loos et al (Loos S 2018), indicating the need for a randomized controlled study in STEC-HUS before a treatment with eculizumab can be recommended. This therapy however seems to be supported by recently published experimental data also suggesting involvement of the complement pathway in the typical HUS form.

The majority of follow-up studies of children with HUS have focused on renal outcome after HUS episode. Data on neurological complications and neurodevelopmental/cognitive outcome in children, but also psychological outcome in children and their parents' after HUS, however, are scarce. Therefore, well-validated long-term data in this field are needed, resulting in the studies described below (original article 1-3).

Original article 1: Neurodevelopmental long-term outcome in children after haemolytic uremic syndrome

Kathrin Buder, Beatrice Latal, Samuel Nef, Thomas J. Neuhaus, Guido F. Laube, Giuseppina Spartà

Pediatr Nephrol. 2015; 30(3):503-13.
(Buder et al 2015)

In this study we recruited 47 children from a sample of 129 patients treated for HUS in the Paediatric Nephrology Unit of the Zurich University Children's Hospital between April 1995 and February 2013. We investigated the long-term neurodevelopmental outcome in children with a history of both typical infection-induced HUS and atypical HUS. Thus, we compared the outcomes of central nervous system involvement (CNS) in HUS.
First, we collected the clinical and demographic data from patients' records, evaluated risk factors and analysed them retrospectively, in a cross-sectional study design. Clinical parameters were obtained from medical records (sex, age at disease, renal function, anuria, requirement of dialysis and CNS involvement during acute phase of HUS) as well as data on other comorbidities and ESRD with renal replacement therapy at follow-up.
Second, we assessed neurodevelopmental outcomes including intellectual and neuromotor performance and conducted a standardized neurological examination.
We examined 47 children with a previous diagnosis of HUS aged between 6 years and 16 years 11 months. The median follow-up was 7.8 years after the first episode of HUS (range 0.4-15.5) and the median age of the children at examination was 10.6 years (range 6-16.9).

This study revealed an overall favourable cognitive outcome, with the intellectual quotient (IQ) of the whole study population falling within the normal range (Wechsler D 2002). However, the neuromotor performance was significantly poorer in the neuromotor performance domains "adaptive fine", "gross motor", "static balance" and "associated movements". Abnormal neurological findings were present in 34% of the children during acute HUS. However, subsequent neurodevelopmental outcome was not significantly different between children with or without CNS involvement. Studies reporting neurodevelopmental outcomes in children after HUS with CNS involvement are scarce. This study focused on the long-term intellectual and neuromotor performance of children after HUS and represents an important step in better defining the long-term prognosis of this severe disease.

Original article 2: Long-term health-related quality of life and psychological adjustment in children after haemolytic-uremic syndrome

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Pediatr Nephrol. 2017; 32(5):869-878.
(Werner et al 2017)

In this paper, a cross-selectional study evaluating long-term in health-related quality of life (HRQoL) and psychological adjustment in children with a history of HUS is described, using a comprehensive single center HUS registry (Buder K 2015 and 2016). Long-term HRQoL and psychological adjustment, defined by behavioural problems, depressive symptoms and posttraumatic stress symptoms, were analyzed in 62 children with a history of HUS. HUS patients are at risk of late and long-term renal and extrarenal complications, such as neurological sequelae, visual disorders and diabetes mellitus (Schifferli A 2010). These events may be very stressful and impact the *patients' HRQoL* and *psychological adjustment*, which are recognized as important outcome measures to evaluate the impact of a disease on an individual patient. While *HRQoL* is a multidimensional concept that focuses on the subjective perception of physical, emotional, social and cognitive dimensions of health, *psychological adjustment* targets the individual's mental health by asking about the presence or absence of behavioural problems and/or psychological symptoms (e.g. depression).

In this study we retrospectively collected medical data from hospital records of the children after a mean of 6.5 years (range 0.1-15.7) post-acute HUS episode. At study follow-up, clinical examination and the laboratory findings were evaluated. In addition, well-validated, multidimensional and standardized questionnaires with reference data, were used to assess HRQoL and psychological adjustment of each child. The average age of the children at the time of study was 9.4 years (range 1.9–16.7). The diagnosis of HUS was classified as (1) typical, infection-mediated HUS, including STEC-HUS and P-HUS, or as (2) atypical HUS (aHUS) based on hereditary and/or acquired disorders of regulation of the alternative complement system. Of the 62 children enrolled in the study 42% had CKD.

The long-term outcome measures assessed, were adapted for child age: *a) in preschool children aged ≤ 6.5 years*, (*proxy report for HRQoL (TAPQOL) and CBCL for psychological adjustment behaviour problems); b) in school-age children, aged > 6.5 years* (*Self-reported and proxy reported for HRQoL (KIDSCREEN), and proxy-reported for psychological adjustment (CBCL), and CDI for Depression and PTSD Reaction Index for posttraumatic stress disorder (proxy reported*)

From these evaluations among the preschool children, parents reported that children were less lively and energetic (HRQoL emotional dimension), but no increased behavioural problems were reported. Among the school-age children, self- and proxy-reported HRQoL was well within or even above the norm, but total behavioural problems were greater. The school-age children reported no increased depression scores. No child met criteria for full or partial HUS-associated posttraumatic stress disorder.

This study was the first to examine HRQoL in paediatric patients with a history of HUS and is therefore a landmark study in the field.

Original article 3: Health-related quality of life and mental health in parents of children with haemolytic uremic syndrome

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Pediatr Nephrol. 2016; 31(6):1035-7.
(Buder et al 2016)

HUS is a life-threatening disease with a mortality of 3-15% during the acute phase. Little is known about health-related quality of life (HRQoL) and mental health of parents having children with a history of HUS. We studied 63 mothers and 58 fathers of a cohort of 63 HUS-affected children. We retrospectively extracted medical data from the children's hospital records. Parental HRQoL, mental health and posttraumatic stress disorder (PTSD) were assessed using standardized self-report questionnaires. The mean time since a child experienced an acute episode of HUS was 6.4 years.

This paper showed that overall most parents of the study sample were doing well in terms of HRQoL and mental health. The HRQoL and mental health of both the mothers and fathers were not impaired compared to normative data.

A small number of parents did however meet criteria for full or partial PTSD diagnosis due to their child's HUS.

This is the first study to report on long-term HRQoL and mental health in parents of HUS-affected children. Its strengths include the use of standardized multidimensional questionnaires and the comparison of results with normative data.

Original article 4: Membranoproliferative glomerulonephritis and C3 glomerulopathy in children: change in treatment modality? A report of a case series

Giuseppina Spartà, Ariana Gaspert, Thomas J. Neuhaus, Marcus Weitz, Nilufar Mohebbi, Urs Odermatt, Peter F. Zipfel, Carsten Bergmann, Guido F. Laube

Clinical Kidney Journal; 2018, 1-12; doi: 10.1093/ckj/sfy006
(Spartà et al 2018)

In this paper the complexity of diagnosis, treatment and variability of outcome in MPGN and C3 glomerulopathy is shown based on a case series of seven children. MPGN and C3 glomerulopathy are rare chronic glomerulonephritis in childhood, leading to renal failure within 10 years in up to 50% of affected children (Cansick JC 2004; Smith RJ 2007). In the past, MPGN was diagnosed and classified by renal histological features into three pathological subtypes. Recently, a link between dysregulation of the alternative complement pathway and the pathogenesis of MPGN was confirmed by findings of mutations in the genes of complement factor proteins in conjunction with repeatedly low serum of complement C3 (Licht C 2007). The histological classification has therefore been reconsidered on the basis of pathogenesis, separating cases into those with glomerular immune deposits staining for immunoglobulins and complement and those characterized by C3 deposition alone (Pickering MC 2013).

This retrospective study evaluated the clinical presentation at disease onset and the outcome of three children with MPGN Type I, three with C3 glomerulonephritis (C3GN) and one with DDD. The children had a median age of 7.3 years at disease onset and were followed for a median of 9 years after diagnosis. All children were screened for the presence of genetic mutations of the alternative complement pathway and clinical, autoimmune data, histological characteristics, GFR, proteinuria, serum complement and biochemical analyses were assessed. Moreover, the benefit of different treatment strategies was analysed.

The evaluation revealed a dysregulation of the complement alternative pathway and mutations/variations in genes of complement-factor-proteins in all children. Nephrotic syndrome at onset was a prognostic unfavourable factor leading to a more severe course, often leading rapidly to ESRD. Only 3 children had a favourable outcome, maintaining a good renal function. Currently there is no established treatment for MPGN and C3G in children or adults. Patients appear to respond differently to various therapy modalities, showing also a great variability independent of histological diagnosis at disease onset.

Treatment with eculizumab has shown promising results in the treatment of some cases of MPGN and C3G (Oosterveld MJ 2015, Vivarelli M 2014). Two patients in this study

experienced a relevant decrease in proteinuria and stabilisation of renal function after treatment with eculizumab.

Treatment regimens and cases series have been reported by others, however, there are not treatment guidelines. The data presented in this study are therefore an important contribution from the clinical- and phenotypic- point of view, adding to accumulating knowledge in the field of genetic and serological investigation in MPGN and C3 glomerulopathy, in order to improve management and to better define more tailored treatment options.

Discussion

A life-threatening disease and its impact on the life of children and their parents

When HUS occurs for the first time in a child, it always represents an incisive event in the life of a family, especially for the parents. The parents mostly report that their child had been healthy before onset of the disease. Therefore, the family is confronted with a sudden, life-threatening disease. The parents are informed that their child can die of HUS and its complications. Because of the severity of the disease, in most cases the child is hospitalized on an intensive care unit. This unforeseeable event can be compared with a sudden trauma (e.g. accident). Often the parents are shocked by the diagnosis, hence, the days following the event are crucial. Frequently, the first question of the parents is: “Why did this happen?”, followed by the questions “How can the disease be treated?” and “What are the consequences?” Therefore, doctors and researchers have the task to try to get to the bottom of these questions.

HUS is a multi-organ disease, and acute renal failure is frequently the first event. About 90% of HUS cases in childhood are infection-induced i.e. they are typical HUS forms, mainly associated with infections caused by Shiga-toxin producing bacteria, usually enterohaemorrhagic *Escherichia coli* (STEC-HUS), but in some regions *Shigella dysenteriae type 1* is also found. Gastroenteritis may therefore often precede HUS. In addition, infections with Streptococcus pneumoniae (P-HUS) and other bacterial and viral agents can trigger HUS (Noris M 2009; Loirat 2012). Only 5-10% of the cases are defined as atypical HUS (aHUS) based on various hereditary and/or acquired disorders of the alternative complement pathway regulation. Extrarenal manifestations are frequent in all forms of HUS and may affect the central nervous system (CNS), gastrointestinal tract, heart, eyes, lungs and skin. CNS involvement represents a major complication associated with increased mortality and risk for neurological sequelae (Siegler RL 1994).

The term HUS encompasses a heterogeneous group of disorders, including STEC-HUS and aHUS. In the latter genetic or acquired dysregulation of the complement alternative pathway is detected in 40-60% of patients. Cobalamin C and DGKE deficiency are two rare metabolic genetic forms of HUS. However, approximately 30% of aHUS arises through unknown mechanisms. Currently it is still debated whether secondary HUS (*figure 2*) should or should not be included in the spectrum of aHUS. Most classifications exclude TMAs or HUS secondary to underlying diseases, labelled as secondary TMAs or secondary HUS, from the spectrum of aHUS. Classifications are still in progress, nevertheless the identification of the mechanisms underlying TMAs has become central in defining HUS (Fakhouri F 2017).

In recent years the majority of follow-up studies in patients with HUS have focused on mortality and renal outcome after a HUS episode (Fremeaux-Bacchi V 2013). In children the results revealed that about 30% of survivors after typical HUS demonstrate long-term renal sequelae (e.g. proteinuria, arterial hypertension, impaired renal function). The mortality rate is higher in atypical HUS (aHUS) and higher in children than in adults (6.7% vs 0.8% at 1 year). However, progression to ESRD after a first episode of aHUS is more frequent in adults than children (46% vs 16%) and a higher severity of acute Illness is strongly associated with a worse long-term prognosis. There is evidence that patients with CNS symptoms (coma seizures, or stroke) and those who need dialysis may have a higher mortality or risk of permanent ESRD at follow up (Garg AX 2003).

However, data reporting on neurodevelopmental outcome in children after HUS are scarce. The study presented in **article 1** was therefore performed to determine the influence of CNS involvement during acute HUS disease on the long-term neurodevelopmental outcome. The study focused on the long-term intellectual and neuromotor performance in children after HUS, including all HUS forms based on the hypothesis that all children with HUS may have a higher risk for adverse neurodevelopmental outcome. The study showed that all patients had an overall favorable neurodevelopmental outcome after a history of HUS, with a normal full-scale IQ. In addition the intellectual performance was not affected by CNS involvement during an acute HUS episode. However, socioeconomic status was positively correlated with full-scale IQ, which is consistent with findings in healthy controls (Largo RH 1989). One-third of the children presented with neurological symptoms during the acute episode of HUS, particularly with seizures and altered consciousness. There are no evidence-based guidelines on the treatment of CNS complications in HUS. Plasmapheresis may benefit some children with severe CNS complications. Recently, Pape et al, (2015) showed, that early use of eculizumab in children with typical HUS and CNS involvement may improve neurological outcomes. However, in severe HUS cases with rapid progression and multiple organ involvement, late treatment with eculizumab has shown less benefit. It is hypothesized therefore that prophylactic therapy with eculizumab, before the development of neurological symptoms, could be advantageous. Recent experimental data suggest involvement of the complement pathway also in typical HUS potentially opening new treatment avenues for typical HUS.

In **article 1,** neurocognitive outcomes were favorable in most children after HUS, however patients who developed ESRD showed a significantly poorer outcome after HUS compared to patients without ESRD. Overall, neuromotor performance was more impaired than IQ particularly fine and gross motor functioning, static balance and movement quality were impaired. Interestingly, motor performance did not differ between children with and without CNS impairment during acute HUS episode. However, 15-38% of the patients had a motor performance below 10% percentile. This poorer motor performance is clinically significant as

children who perform below the 10th percentile often have difficulties participating in activities of daily life and demonstrate poorer hand writing skills and slower speed. The pathophysiologic mechanisms leading to impaired neuromotor outcome after HUS remain to be elucidated. In addition to cerebral thrombotic microangiopathy and uremia, other factors, such as long hospital stay, more parental protectiveness and less experience may contribute to adverse neuromotor performance as described in other cohorts of paediatric patients with various diseases (Schaefer C 2013).

It is therefore plausible that an HUS event may have an important impact on the quality of life of children and their parents, as shown in **article 2 and article 3**. HUS patients are at risk for late and long-term renal and extra-renal complications. This situation may be very stressful for patients and their parents. We hypothesized a negative impact of the disease on patients' and parents' health-related quality of life (HRQoL). The results indicate that the patients' HRQoL was comparable to normative data. However, a few parents suffered from full or partial posttraumatic stress disorders (PTSD) due to their child's HUS.

Overall HRQoL of the children with a history of HUS was not impaired, as shown in **article 2**. As there are no prior studies on this topic, no comparison with the literature was possible. **In article 2**, the parents of pre-school children rated their child's *HRQoL* as similar - or even better- than a normal control group. This may be explained by the possibility that they rated their child's current HRQoL in comparison to that of when their child was sick. These results are in contrast to those of other studies indicating lower HRQoL in paediatric patients with chronic kidney disease (CKD) compared to healthy controls. Indeed in our study, the rate of CKD was 42%, therefore lower than in other studies, which may explain the better HRQoL. However, our study is in line with Askenazi et al (Kidney Int 2006) who observed no difference in long-term HRQoL in children 3-5 years of age after acute renal failure of various causes. Thus, in our study, children with a history of HUS, even when severe, appear to recover quite well over the long-term, and the patient's acute medical characteristics (e.g. longer length of stay in ICU in severe case) have a minor impact on long-term HRQoL. Furthermore, the data revealed, that neither medical characteristics during an acute episode nor the presence of CKD were significantly associated with impaired HRQoL reported by the parents in pre-school children. In contrast, a lower self-reported HRQoL was observed among school-age children in association with the presence of CKD. Regarding the *psychological adjustment*, the parents reported increased total behavioral problems among school-age children. This is in contrast to other studies which found no clinically significant behavioural problems in children with a history of HUS (Schlieper A 1999). These difference may be explained by the longer period since acute HUS in the CKD patients and the high number of children affected by CKD. Thus, school-age children with a history of HUS may be at risk of poorer psychological adjustment. A longer stay in ICU as well as longer periods of dialysis and hospital stay were significantly

associated with more parent-reported total behavioural problems. Thus, these medical characteristics can be seen as risk factors for long-term psychological maladjustment in children with a history of HUS. None of the school-age children in our cohort perceived HUS as a traumatic event or met criteria for full or partial PTSD. This in contrast to the parents, some of whom met the criteria for full or partial PTSD (Buder K 2016) as revealed **in article 3**. Therefore, knowledge of parents' impairment in mental health and quality of life are also important, as it may impact the child's adjustment to the disease. Thus, it has been shown that parents with increased problems may be too absorbed in regulating their own feelings to be able to provide sensitive support for their child. Findings in **article 3** showed that HRQoL and mental health were not impaired in the mothers and fathers compared to normative data, although a full, HUS-related PTSD was diagnosed in two fathers but no mothers. Overall, parental HRQoL was comparable to or even greater than the population norm, which is consistent with findings from a number of previously published studies on HRQoL in parents of children with other life-threatening or chronic diseases (e.g. meningococcal septic shock, Kawasaki syndrome or juvenile polyarthritis). Interestingly, this differs from findings in parents of children with ESRD, where HRQoL was lower than that of parents of healthy controls (Hatzmann J 2008). One possible explanation for the findings in **article 3** is the small number of children with ESRD or advanced CKD. Another possible explanation for the positive results of our study may be that the parents of HUS-affected children rate their own physical health as better than the norms because they rate their health in comparison to that of their sick child. Also, a shorter time since an acute episode of HUS was a significant predictor of lower maternal HRQoL, which is consistent with findings in mothers of children having other chronic disease (e.g. heart disease). No significant association between HUS–related or sociodemographic characteristics and parental mental health was observed.

TMA and HUS as a link to other glomerulopathies

Based on recent advances in knowledge regarding the clinical and pathophysiological aspects of TMA, especially in HUS, interest has increased in glomerulopathies and other renal diseases where complement involvement was suspected e.g. because of low complement C3, haemolysis or thrombocytopenia. The finding of one or more common denominators in TMA with different diseases has led to growing research in the field of complement diseases.

As described in **article 4**, in membranoproliferative glomerulonephritis (MPGN) and C3-glomerulopathy (C3G), the hereditary and acquired complement defects are similar, although subtly different to those seen in complement-mediated aHUS, and it is perhaps not surprising that concurrent and sequential manifestation of C3G and TMA have been reported (Manenti L 2013; Cooper M 2004). Mutations in CFH are observed in both complement-mediated aHUS and C3G; the reason for this genetic pleiotropy is not fully understood, but the location of the

mutation within the gene may be important. In aHUS, the majority of the mutations are located at the C-terminal of FH, which binds to C3b and glycosaminoglycans on host cells to mediate cell surface protection, whereas in C3G, mutations are more often located at the N-terminal of FH, which mediates complement regulation in the fluid phase. Studies from different small case series revealed that clinical presentation and the measurement of plasma C3, C3d and sC5b-9 do not allow differentiation between C3G and MPGN.

We observed similar findings in our case series of 7 children described in **article 4**. Genetic analysis revealed a dysregulation of the alternative complement pathway and mutations/variation in genes of complement factors proteins in all 7 children with MPGN, C3 glomerulonephritis and dense deposit disease (DDD). Three patients had a favorable outcome: two patients with MPGN I and one with C3GN, the latter without any genetic variation in CFHR proteins. Five of seven children had a heterozygous mutation/deletion or variation in CFHR proteins 1, 2, 3 or 5. Genetic alterations, including variations or polymorphisms could at least in part explain the different outcomes and responses to various treatment modalities (e.g. renin-aldosteron-angiotensin-receptor-blockers, immunosuppressive drugs, eculizumab, plasmapheresis). There is currently no established treatment for these diseases. Recently, treatment with eculizumab, a monoclonal antibody binding to C5 of the alternative pathway, has shown promising results in the treatment of some cases of MPGN and C3G. Two patients in our series, one with C3GN and one with MPGN I, exhibited a significant decrease in proteinuria in their native kidney after eculizumab, but elevated activity of the alternative pathway persisted. Consistent with observations of other authors treating MPGN, this finding suggests that eculizumab is not completely effective in inhibiting sC5b-9 activity in C3G, and it is suspected that sC5b-9 alone may not reflect disease activity.

Secondary atypical HUS has been described in association with infective agents, including HIV. Other causes identified for secondary HUS include connective tissues diseases, pregnancy and post-transplantation associated TMA. Several drugs have also been reported to induce TMA. TMA can also occur in association with IgA nephropathy, ANCA-associated vasculitis, membranous nephropathy, FSGS, although it may be a histopathological finding without biochemical or clinical manifestation (Brocklebank V 2017).

In the view of the heterogeneity in pathophysiological, clinical and genetic presentation of TMA/HUS-associated diseases, new treatment therapies are under development and will hopefully become available in the next years in order to optimize treatment for these complex diseases.

Conclusions

The main goal of the studies reported here was to improve understanding of the management and long-term outcome in children with HUS. Children and adolescents with HUS have a

normal intellectual outcome, but a significant impairment in motor function. Neurological complications during the acute episode of HUS were not associated with a poorer neurodevelopmental outcome. Therefore, long-term observation of all children after HUS is advisable for the early detection of neurodevelopmental deficits. Healthcare providers should also be especially alert for any signs of behavioural problems in school-age children with a history of HUS and for a lower HRQoL in the preschool children. Early detection of any of these abnormalities could permit interventions that may minimize the long-term impact. Similarly, healthcare providers should pay special attention to parents' reports of PTSD symptoms during the clinical follow-up of a child with HUS. Although the prevalence of HUS-related PTSD seems to be small, even partial PTSD might lead to functional impairment and some parents would benefit from psychological support. Therefore, knowledge of the impairments in parental HUS-related PTSD might help to identify those aspects of the clinical experience which most strongly affect the parents to permit better parental support during the acute illness, and, if necessary, to enable these parents to be monitored closely and to get access to psychological support.

Methodological differences and the small number of patients in single-center studies make it difficult to compare findings in the literature. Therefore, it would be advisable to perform multicenter-studies in order to optimize generalizability of the results. Based on previous studies, investigation of HRQoL and mental health in other caregivers (e.g. siblings, grandparents) may be of importance in families of children after an HUS-event.

Concerning the pathophysiology of TMA and HUS, many questions remain still open and need to be clarified. There is evidence of complement activation (e.g. low C3 plasma levels and tissue staining) in many TMAs, but whether this is a primary event, a disease modifier, or a bystander phenomenon has not yet been definitively established. In addition, the relative roles of effector molecules (e.g.C5a, C5b-9) of the alternative pathway in causing disease also remains to be established.

In a clinical setting, it is relevant to consider counseling of family members and to perform genetic tests, which are cost-effective and easily accessible, in order to provide early and more rapidly diagnosis of TMA-associated diseases. This approach would support genetic screening over a larger area and increase knowledge concerning TMA-associated diseases as well as permit appropriate tailoring of treatment-guidelines.

Our results strongly support the need for a formal transition from paediatric to adult nephrology care especially after HUS, as renal (and extrarenal) long-term complications after HUS in childhood (e.g. proteinuria, hypertension, renal insufficiency) can occur late in adult life.

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APPENDIX: PUBLICATIONS CONSTITUTING THE CUMULATIVE HABILITATION

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ORIGINAL ARTICLE

Neurodevelopmental long-term outcome in children after hemolytic uremic syndrome

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Abstract
Background To investigate the long-term neurodevelopmental outcome in children after hemolytic uremic syndrome (HUS) and to compare outcome dependent on central nervous system (CNS) involvement during HUS.
Methods A single-center retrospective cohort of 47 children was examined at a median age of 10.6 (range 6–16.9) years and a median follow-up of 7.8 (range 0.4–15.3) years after having had HUS. Intellectual performance was assessed with the German version of the Wechsler Intelligence Scale 4th version and neuromotor performance with the Zurich Neuromotor Assessment (ZNA). The occurrence of neurological symptoms during the acute phase of HUS was evaluated retrospectively.
Results Mean IQ of the whole study population fell within the normal range (median full scale IQ 104, range 54–127). Neuromotor performance was significantly poorer in the domains "adaptive fine," "gross motor," "static balance" (all $p<0.05$) and "associated movements" ($p<0.001$); only the "pure motor" domain was within the normal reference range. Neurological findings occurred in 16/47 patients (34 %) during acute HUS. Neurodevelopmental outcome was not significantly different between children with or without CNS involvement.
Conclusions Our follow-up of children after HUS showed a favorable cognitive outcome. However, neuromotor outcome was impaired in all study participants. Neurological impairment during acute HUS was not predictive of outcome.

Keywords Intellectual · Motor · Neurocognitive outcome · Central nervous system involvement · Hemolytic uremic syndrome

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Introduction

Hemolytic uremic syndrome (HUS) is a multi-organ and life-threatening disease characterized by hemolytic anemia, thrombocytopenia and acute renal injury. HUS is also one of the most frequent causes of acute renal failure in childhood [1] and may result in long-term renal and extrarenal sequelae [2–5].

About 90 % of HUS cases in childhood are infection-induced, i.e. they are typical HUS forms, mainly mediated by infections caused by Shiga toxin-producing bacteria, usually enterohemorrhagic *Escherichia coli* (STEC-HUS) but in some regions *Shigella dysenteriae* type 1. In addition, infections with *Streptococcus pneumoniae* (P-HUS) and other bacterial and viral agents can trigger HUS [6, 7]. Only 5–10 % of cases are defined as atypical HUS (aHUS) based on various hereditary and/or acquired disorders of the alternative complement pathway regulation [6–8]. Renal replacement therapy at disease onset is required in up to 65 % of STEC-HUS patients [9], 84 % of those with P-HUS [10] and 59 % of aHUS patients [5].

Extrarenal manifestations are frequent in all HUS forms, including STEC-HUS [11, 12], P-HUS [13, 14] and aHUS [5, 15], and may affect the central nervous system (CNS), gastrointestinal tract, heart, eyes, lungs, parotid glands and skin.

CNS involvement represents a major complication that is associated with increased mortality [2, 11] and risk for neurological sequelae [16].

Studies reporting on neurodevelopmental outcome in children after HUS are scarce, and the results suggest a normal neurocognitive outcome [17–19]. However, a trend towards impaired full-scale and verbal comprehension IQ in these children has also been described [17]. Data on neuromotor outcome are limited to information on impaired fine motor skills in children with a history of HUS and severe CNS involvement [18].

In the study reported here, we focused on the long-term intellectual and neuromotor performance in a single-center cohort of children after HUS, including typical and atypical HUS forms. The hypothesis was that all children with HUS may have a higher risk for adverse neurodevelopmental outcome. Furthermore, the study was performed to determine the influence of CNS involvement during acute HUS disease on the long-term neurodevelopmental outcome.

Methods

Patients

The study cohort consisted of 47 children (22 males, 25 females; median age 10.6 years, age range 6–16.9 years) with a history of both typical infection-induced HUS and atypical HUS. The neurodevelopmental testing was part of a comprehensive single-center study on long-term renal outcome, psychological adjustment and quality of life in HUS patients. The study was approved by the Cantonal Ethics Committee Zurich and registered at ClinicalTrials.gov (NCT 01666548). Written informed consent was obtained by the parents and by the adolescents themselves if they were ≥15 years. Inclusion criteria for neurocognitive and neuromotor assessment were: (1) previous diagnosis of HUS and (2) age between 6 years and 16 years 11 months during the study period between February 2012 and February 2013.

HUS was defined as non-immunological hemolytic anemia (hemoglobin<100 g/l), thrombocytopenia (thrombocytes <150.000/μl) and features of acute renal injury (plasma creatinine elevation above the age-related norm range; proteinuria, hematuria or renal ultrasound abnormalities). Two of the enrolled patients—one with STEC-HUS requiring dialysis and one with recurrent aHUS due to complement factor H mutation—did not meet the criteria for thrombocytopenia. The diagnosis of HUS in all patients was confirmed by pediatric nephrologists. Based on the different approaches used in published studies to classify HUS [20–22] we categorized the disease as (1) typical, infection-induced HUS, including STEC-HUS and P-HUS, and (2) aHUS based on currently proposed HUS nomenclature [21].

The age criterion of 6–16 years was used to study long-term neurocognitive outcome using one intellectual test, namely, the German version of the *Wechsler Intelligence Scale 4th version* [23].

Participants were recruited from a sample of 129 patients treated for HUS at the Pediatric Nephrology Unit of Zurich University Children's Hospital between April 1995 and February 2013. Seven patients died during an acute episode of HUS, five patients were lost to follow-up and 42 patients did not fulfil the age criterion (26 were aged <6 years and 16 were aged≥17 years). Thus, 75 patients were eligible for the study. Twenty-six parents or children refused to participate; two additional patients were excluded due to a pre-existing neurodevelopmental impairment resulting from trisomy 21 in one and an unclassified syndrome in another. The final study cohort included 47 (63 %) of the children originally eligible for entry. Demographic and clinical characteristics did not differ significantly between enrolled patients and those not enrolled in terms of sex, HUS form, socioeconomic status, age at diagnosis of HUS, frequency of neurological complications during the acute phase of HUS, occurrence of anuria, need for dialysis during the acute phase of HUS, length of hospital stay, estimated glomerular filtration rate (eGFR) at time of discharge, need of dialysis at time of discharge and development of end-stage renal disease (ESRD).

The clinical and demographic data needed to evaluate potential risk factors were extracted from patients' records and analyzed retrospectively. Values for the following parameters were obtained from the medical records: sex, age at disease onset, renal function, anuria defined as urine output<0.2 ml/kg per hour, requirement of dialysis and CNS involvement during the acute episode of HUS. CNS involvement was defined as presence of neurological findings including seizures, altered consciousness, ataxia, muscle tone abnormalities, hemiplegic symptoms, dysarthria, visual disorders, movement disorders and vestibular symptoms. Since conditions such as anemia or dehydration may affect mental status, CNS involvement was only considered if the clinical symptoms were severe and not attributable to an underlying non-cerebral medical condition. None of the studied children had a neurological disease prior to HUS.

Other comorbidities and ESRD with renal replacement therapy at follow-up were also recorded. Renal function was evaluated by eGFR, expressed in millimeters per minute per 1.73 m^2, according to the Schwartz formula using the local factor k of 40 for all children and by the plasma creatinine concentration (in μmol/l) [24]. Information on additional potential neurological risk factors and interventions performed since HUS was retrieved from parental interviews at the time of neurodevelopmental assessment.

Neurodevelopmental outcome assessment

The neurodevelopmental outcome assessment included as assessment of intellectual and neuromotor performance and a standardized neurological examination [25]; both were performed at the Child Development Center of Zurich University Children's Hospital by one experienced developmental pediatrician. Socioeconomic status was estimated based on maternal education level and paternal occupation using an education scale ranging from 2 to 12, with 2 being the lowest and 12 the highest education score [26].

Intellectual performance

Of the 47 participants, 46 were assessed using with the German version of the *Wechsler Intelligence Scale 4th version* [23]. This test provides IQ subscales for verbal comprehension, perceptual reasoning, working memory and processing speed, which together form the full-scale IQ. One 9-year-old patient with P-HUS associated with meningitis and serious neurological complications was not able to perform the *Wechsler Intelligence Scale 4th version* and was examined using the German version of the *Wechsler Preschool and Primary Scale of Intelligence 3rd version* [27].

Neuromotor performance

Neuromotor performance was examined with the Zurich Neuromotor Assessment (ZNA), a standardized, videotaped test for children aged 5 to 18 years which is used to investigate specific motor skills based on timed performances and movement quality [28, 29]. The ZNA contains five block components including: (1) pure motor domain, (2) adaptive fine motor domain, (3) adaptive gross motor domain, (4) static balance and (5) associated movements. The results are expressed as *z*-scores, i.e. the standard score of the reference population based on age and sex.

Statistical analysis

Statistical analysis was performed with SPSS for Windows version 20.0 and 22.0 (IBM Corp,, New York, NY). Differences between participants' data and normative data were calculated using the univariate *t* test, and differences between subgroups were assessed using the Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables. Multivariate linear regression was conducted to evaluate the association between risk factors and full-scale IQ scores. Variables included in the regression model were socioeconomic status, duration of hospital stay, CNS involvement and eGFR at time of discharge. Two children with very low full-scale IQ scores (54 and 62) were excluded for the multiple regression analysis in order to comply with the requirements of a normal distribution in the study sample. A p value of<0.05 was considered to be statistically significant.

Results

Sample description

Forty-seven patients (22 boys and 25 girls; median age 10.6 years, range 6–16.9 years) with a history of STEC–HUS (n=38), P-HUS (n=6) and aHUS (n=3) and a median follow-up after HUS of 7.8 (range 0.4–15.3) years participated in this study (for detailed information on each participant, see Table 1).

Of the 38 STEC-HUS patients, 24 tested positive for Shiga toxin. Genetic analysis of the three aHUS patients revealed one or more mutations of complement-related factors. The median age at onset of HUS was 1.8 (range 0.3–14.4) years. Thirty-three children (70 %) required acute renal replacement therapy combined with either peritoneal dialysis (n = 24 patients), hemofiltration or hemodialysis (n = 6) or a combination of both treatment modalities (n = 3). At time of discharge the median eGFR was 54 (range 13–178) ml/min per 1.73 m^2. Forty-one (87 %) patients had an impaired eGFR defined as<90 ml/min per 1.73 m^2. One patient was on dialysis when discharged and remained on dialysis for 127 days, subsequently progressing to ESRD. Five patients developed ESRD, of whom four underwent renal transplantation (RTPL) (Table 1).

The median eGFR at neurodevelopmental testing—excluding the four children who underwent RTPL—was 113 (range 12–178) ml/min per 1.73 m^2; ten of these children had impaired renal function with an eGFR of<90 (range 12–88) ml/min per 1.73 m^2. Two of the four patients undergoing RTPL had good renal graft function defined as an eGFR of>60 (respectively 92 and 163) ml/min per 1.73 m^2, while the remaining two children showed impaired graft function (47 and 54 ml/min per 1.73 m^2, respectively). The children with CNS involvement has a significantly lower median eGFR at both discharge and follow-up (46 and 89 ml/min per 1.73 m^2, respectively; p=0.014) than the children without CNS involvement (83 and 126 ml/min per 1.73 m^2, respectively; p=0.004) (Table 2).

CNS involvement during acute episode of HUS

Sixteen children (34 %) presented with CNS involvement during the acute episode of HUS with a broad spectrum of neurological symptoms, consisting predominantly of seizures (12/16) or altered consciousness (7/16) (Table 3).

In the STEC-HUS group neurological symptoms were observed in 12 of 38 patients. Two children received treatment with plasmapheresis due to severe neurological complications (Table 1). Four of the six P-HUS patients presented with neurological symptoms, including two with pneumococcal meningitis. None of the patients with aHUS manifested CNS

Table 1 Characterization of all 47 study participants according to hemolytic uremic syndrome form

Patient	Diagnosis[a]	Neurological symptoms during acute HUS	Radiological findings during acute HUS[b]	Additional neurological risk factors	Full scale IQ (*Wechsler Intelligence Scale 4th version*)	Renal outcome (eGFR; ml/min per 1.73 m^2)[c]
1	STEC-HUS	Seizure	CCT: normal	–	110	113
2	STEC-HUS	–	NI	Attention deficit hyperactivity disorder	88	167
3	STEC-HUS	–	NI	–	99	95
4	STEC-HUS	–	NI	–	108	126
5	STEC-HUS	–	NI	–	104	111
6	STEC-HUS	–	NI	–	104	102
7	STEC-HUS	Seizure	CCT: normal	–	98	88
8	STEC-HUS	Seizure	NI	–	109	133
9	STEC-HUS	–	NI	–	102	172
10	STEC-HUS	–	NI	–	112	79
11	STEC-HUS	Seizure	CCT: normal	–	105	100
12	STEC-HUS	Seizure	NI	Resuscitation (mechanical)	105	139
13[d]	STEC-HUS	Seizure, ataxia, movement disorder, dysarthria	CCT, CMRI: basal ganglia infarctions/ischemias	–	108	53
14	STEC-HUS	–	NI	–	101	105
15	STEC-HUS	Altered consciousness	NI	–	104	78
16	STEC-HUS	–	NI	–	101	135
17	STEC-HUS	Seizure, altered consciousness	CCT: not specific	–	105	74
18	STEC-HUS	Seizure, altered consciousness	CCT: normal; CMRI: multiple infarctions	–	103	12 (ESRD)
19	STEC-HUS	–	NI	–	115	112
20	STEC-HUS	–	NI	–	105	178
21	STEC-HUS	–	NI	–	105	131
22	STEC-HUS	–	NI	–	118	171
23	STEC-HUS	–	NI	–	84	47 (Status after RTPL)
24	STEC-HUS	–	NI	–	108	103
25	STEC-HUS	–	NI	–	103	175
26	STEC-HUS	–	NI	–	127	94
27	STEC-HUS	–	NI	–	99	54 (Status after RTPL)
28	STEC-HUS	–	NI	–	99	147
29	STEC-HUS	–	NI	–	102	83
30	STEC-HUS	–	NI	–	121	133
31	STEC-HUS	–	NI	Prematurity (31 4/7 gestational age)	82	126
32	STEC-HUS	–	NI	–	109	107
33[d]	STEC-HUS	Seizure, altered consciousness, visual disorder, ataxia, vestibular symptoms	CCT, CMRI: normal	–	127	72

Table 1 (continued)

Patient	Diagnosis[a]	Neurological symptoms during acute HUS	Radiological findings during acute HUS[b]	Additional neurological risk factors	Full scale IQ (*Wechsler Intelligence Scale 4th version*)	Renal outcome (eGFR; ml/min per 1.73 m^2)[c]
34	STEC-HUS	–	NI	–	102	139
35	STEC-HUS	–	NI	–	96	141
36	STEC-HUS	–	NI	–	110	128
37	STEC-HUS	Altered consciousness	NI	–	105	90
38	STEC-HUS; sepsis due to *Streptococcus pneumoniae*	Seizure, muscle tone abnormality	CCT: not specific	–	100	72
39	P-HUS (pneumonia)	Hemiplegic symptoms	CCT: normal; CMRI: leukoencephalopathy	–	75	92 (Status after RTPL)
40	P-HUS (meningitis)	Seizure, hemiplegic symptoms	CCT: subdural empyema, ischemic changes, hydrocephalus	Sensorineural hearing loss, cochlear implant	90	116
41	P-HUS (pneumonia)	–	NI	–	75	146
42	P-HUS (pneumonia, peritonitis)	–	NI	–	109	129
43	P-HUS (pneumonia)	Ataxia, altered consciousness	CCT: not specific	–	91	141
44	P-HUS (meningitis)	Seizure, ataxia, altered consciousness, muscle tone abnormality	CCT: meningoencepha-litis, subdural hygroma; CMRI: leukoencephalo-malacia, hydrocephalus internus, signs of intra-cranial hypertension	Ventriculoperitoneal shunt implant, sensorineural hearing loss, cochlear implant, transient symptomatic epilepsia	62[f]	77
45	aHUS (CFH mutation, one clinical relapse,no complement activity)	–	NI	–	125	126
46[e]	aHUS (DEAP-HUS, ongoing complement activity)	–	NI	–	126	113
47	aHUS (combined MCP and CFI mutation, one clinical relapse leading to ESRF)	–	NI	Resuscitation (mechanical & medical), viral meningitis	54	163 (Status after RTPL)

[a] HUS, Hemolytic uremic syndrome; STEC-HUS, *Escherichia coli* hemolytic uremic syndrome; P-HUS, *Streptococcus pneumoniae* hemolytic uremic syndrome; aHUS, atypical hemolytic uremic syndrome; DEAP-HUS, deficiency of complement factor H-related plasma proteins and autoantibody-positive form of hemolytic uremic syndrome; MCP, membrane cofactor protein CD46; CFH, complement factor H; CFI, complement factor I; ESRF, end-stage renal failure

[b] CCT, Cerebral computed tomography; CMRI, cerebral magnetic resonance imaging; NI, not investigated

[c] eGFR, Estimated glomerular filtration rate; RTPL, renal transplantation

[d] Treatment with plasmapheresis during acute HUS

[e] Treatment with plasmapheresis because of complement factor H antibodies (no cerebral impairment)

[f] The patient was not able to perform the *Wechsler Intelligence Scale 4th version* and was therefore examined with the German version of the *Wechsler Preschool and Primary Scale of Intelligence 3rd version*

Table 2 Demographic and clinical characteristics of the 47 hemolytic uremic syndrome (HUS) patients enrolled in the study

Demographic and clinical characteristics	CNS involvement during acute episode of HUS (n=16)	No CNS involvement during acute episode of HUS (n=31)	p value
General data			
Sex: male/female (n)	6/10	16/15	0.54
HUS-classification (n)			
STEC-HUS	12	26	
P-HUS	4	2	
aHUS	0	3	
Socioeconomic status score	8 (7–12)	8 (2–12)	0.38
Acute episode of HUS			
Age (years)	1.3 (0.3–14.4)	2.2 (0.4–13.3)	0.24
Anuria (n)	14	14	0.006*
Duration of anuria (days)	8 (1–46)	9 (2–20)	0.58
Dialysis (n)	14	19	0.09
Duration of dialysis (days)	13 (5–79)	11 (3–23)	0.18
Mode of dialysis			
Peritoneal dialysis (n)	9	14	
Hemofiltration/hemodialysis (n)	4	2	
Combination of peritoneal dialysis and hemofiltration/hemodialysis (n)	1	3	
Duration of hospital stay (days)	26 (10–97)	16 (5–54)	0.030*
eGFR at discharge (ml/min per 1.73 m^2)	46 (13–125)	83 (14–178)	0.014*
Dialysis at discharge (n)	1	0	0.34
Follow–up			
Age (years)	11.1 (6.3–16.3)	10.4 (6.0–16.9)	0.50
Time interval HUS to follow-up (years)	9.1 (0.6–15.3)	7.2 (0.4–15.1)	0.27
Development of ESRD (n)	2	3	1.00
Duration of dialysis in total (acute and chronic) (days)	13 (5–218)	12 (3–1560)	0.65
Status after RTPL (n) n	1	3	1.00
eGFR at neurodevelopmental examination (ml/min per 1.73 m^2)	89 (12–141)	126 (47–178)	0.004*

*Significant difference at $p< 0.05$

Results are presented as the median with the range in parenthesis

CNS, Central nervous system; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; RTPL, renal transplant; STEC-HUS, *Escherichia coli* hemolytic uremic syndrome; P-HUS, *Streptococcus pneumoniae* hemolytic uremic syndrome; aHUS, atypical hemolytic uremic syndrome

involvement, but one was resuscitated due to respiratory failure following RTPL.

Neuroimaging studies were performed in 12 of 16 patients with CNS involvement (cerebral computed tomography in 7, cerebral magnetic resonance imaging in 5 and both investigations in 5 children), revealing cerebral abnormalities in five patients: two with cerebral infarctions (both STEC-HUS), two with meningitis-associated complications and one child with leukoencephalopathy (P-HUS) (Table 1).

Comparison of clinical and demographic characteristics between participants without and with CNS involvement during the acute episode of HUS showed that anuria (p=0.006), longer duration of hospital stay (p=0.03) and impaired eGFR, both at discharge (p=0.01) and at time of neurodevelopmental testing (p=0.004), were significantly more common in patients with CNS impairment (Table 2).

Additional comorbidities, not HUS related and potentially leading to neurodevelopmental impairment, were present in four children, with one child each having status after viral meningitis and resuscitation for pulmonary edema after RTPL, resuscitation associated with an anesthetic accident, prematurity and attention deficit hyperactivity disorder treated with methylphenidate, respectively. Two additional children with P-HUS developed severe cerebral complications (Table 1): subdural empyema and hydrocephalus, respectively.

Intellectual performance

The median full-scale IQ of the study cohort was normal with a value of 104 {54–127 points; comparison to norm of 100 [±15=1 standard deviation (SD)]: p=0.39}. All subscales were in the normal range: verbal comprehension [102 (range

Table 3 Neurological symptoms of the 16 patients enrolled in the study with CNS involvement during acute HUS

Neurological symptoms	Frequency
Seizures	12/16
Isolated seizures	5/16
Altered consciousness	7/16
Isolated altered consciousness	2/16
Ataxia	4/16
Muscle tone abnormality	2/16
Hemiplegic symptoms	2/16
Dysarthria	1/16
Visual disorders	1/16
Movement disorders	2/16
Vestibular symptoms	1/16
>1 neurological symptoms	8/16
Number of neurological symptoms	
1	8/16
2	5/16
3	0/16
4	1/16
5	2/16

Results are presented as the number of patients

CNS, Central nervous system; HUS, hemolytic uremic syndrome

69–130); *p*–0.57], working memory [102 (54–144); *p*=0.52], processing speed [100 (65–129); *p*=0.69] and perceptual reasoning [108 (61–129); *p*–0.03].

Six children (13 %) showed a full-scale IQ of<85 (−1 SD). Two of these two patients had a full-scale IQ of<70 (−2 SD)—one with P-HUS and pneumococcal meningitis and multiple complications requiring ventriculoperitoneal shunt and cochlear implant and the second with a past history of resuscitation episode, aHUS and ESRD in infancy (Table 1).

Children with a history of ESRD showed a poorer neurocognitive outcome than children without ESRD in terms of verbal comprehension [88 (range 69–95) vs. 105 (79–130); *p*=0.004], working memory [87 (54–102) vs. 102 (74–144); *p*=0.008] and full-scale IQ [84 (54–103) vs. 105 (62–127); *p*=0.010].

There were no significant differences between the 16 individuals with and the 31 individuals without CNS involvement during the acute phase of HUS (Table 4). Socioeconomic status did not differ between these two groups (Table 2). Furthermore, the exclusion of patients with neurodevelopmental comorbidities (*n*=6) and those with development of ESRD (*n*=5) did not significantly alter the results of the intellectual outcome.

Neuromotor performance

Forty-seven children (22 boys, 25 girls) performed the ZNA. Except for the pure motor domain, all other domains of the neuromotor performance were significantly impaired compared to the normal controls (Table 5). Between 15 and 38 % of the children performed poorer than the 10th percentile within the five ZNA domains (Table 5).

Table 4 Intellectual performance[a] of the 16 children with and 31 children without CNS involvement during the acute episode of HUS (*n*=47)

Full-scale and subscale IQ	CNS involvement during acute episode of HUS (*n*=16) [b]	No CNS involvement during acute episode of HUS (*n*=31)	*p*-value
Full-scale IQ	105 (62–127)[b]	104 (54–127)	0.62
Verbal comprehension index	99 (81–124)	103 (69–130)	0.49
Similarities	11 (6–14)	12 (4–17)	0.51
Vocabulary	10 (5–15)	10 (3–14)	0.76
Comprehension	9 (6–16)	10 (7–19)	0.31
Perceptional reasoning index	108 (81–117)	108 (61–129)	0.81
Block design	12 (5–16)[b]	12 (5–18)	0.74
Picture concepts	10 (6–14)	10 (2–13)	0.84
Matrix reasoning	11 (8–14)	11 (2–18)	0.90
Working memory index	102 (56–135)	102 (54–144)	0.98
Digit span	10 (3–16)	10 (3–16)	0.78
Arithmetic	10 (2–17)	11 (1–19)	0.60
Processing speed index	96 (71–129)[b]	103 (65–129)	0.29
Coding	9 (4–14)[b]	10 (4–14)	0.40
Symbol search	10 (5–16)[b]	12 (3–16)	0.40

Results are presented as the median IQ score, with the range in parenthesis

CNS, Central nervous system; HUS, hemolytic uremic syndrome

[a] Intellectual performance was assessed using the *Wechsler Intelligence Scale 4th version*

[b] One patient (Table 1, patient no. 44) was not able to perform the *Wechsler Intelligence Scale 4th version* except the subtests "Block design", "Coding" and "Symbol Search.. His results are only included in the subtests, processing speed index and full scale IQ

Table 5 Motor performance[a] data of all patients (n=47) and of the 16 children with and 31 children without central nervous system (CNS) involvement during the acute episode of hemolytic uremic syndrome (HUS)

Zurich Neuromotor Assessment domains	All patients			CNS involvement during acute episode of HUS (n=16)	No CNS involvement during acute episode of HUS (n=31)	p value[d]
	z-score	<P10[b]	p value[c]			
Timed performances						
Pure motor	0.10 (−5.5 to 4.6)	15 % (7/47)	0.73	0.25 (−5.5 to 1.7)	0.10 (−2.4 to 4.6)	0.65
Adaptive fine motor	−0.30 (−3.6 to 3.5)	28 % (13/46)	0.042*	−0.23 (−2.7 to 1.2)	−0.30 (−3.6 to 3.5)	0.72
Adaptive gross motor	−1.00 (−7.5 to 2.5)	36 % (16/45)	0.003*	−1.30 (−7.5 to 2.2)	−1.00 (−3.4 to 2.5)	0.19
Static balance	−0.25 (−3.0 to 1.7)	17 % (8/46)	0.007*	−0.20 (−3.0 to 0.6)	−0.30 (−3.0 to 1.7)	0.84
Associated movements	−1.10 (−3.2 to 2.0)	38 % (18/47)	<0.001*	−1.25 (−3.2 to 0.2)	−0.90 (−2.3 to 2.0)	0.10

*Significant difference at $p< 0.05$

Results are presented as the median z-score with the range in parenthesis

[a] Motor performance was assessed using the Zurich Neuromotor Assessment (ZNA)

[b] <P10 indicates the proportion of patients presenting with z-scores of<−1.282 (i.e. results<10th percentile)

[c] p value calculated for z-score difference to norm

[d] p value calculated for z-score difference between patients with and without CNS involvement

When participants with additional neurodevelopmental comorbidities were excluded, the neurodevelopmental outcome compared to normal controls was still impaired except for the pure motor and the adaptive fine motor domain (p>0.07). Participants with a history of ESRD (n=5) had significantly poorer results in the domain static balance than those without ESRD [−1.9 (range −3.0 to −0.7) vs. −0.2 (−3.0 to 1.7); p= 0.003].

Motor therapies (including psychomotor, physical and ergotherapy) were reported for nine children (19 %). There were no significant differences between children with and without CNS involvement in terms of frequency of motor therapies (6/31 vs. 3/16, respectively; p=0.64).

Neurodevelopmental outcome in children with STEC-HUS

Table 6 presents the developmental outcome for children with only STEC-HUS—which was the commonest HUS form present in the study cohort (n=38). Compared to normal controls, children with STEC-HUS showed a favorable intellectual outcome. In contrast, neuromotor outcome was impaired in the ZNA domains “adaptive gross motor” and “associated movements”. In these domains, 34 % and 39 % respectively performed poorer than the 10th percentile (Table 6).

Prognostic factors

Potential risk factors for poorer IQ were evaluated in a multivariate linear regression analysis . Socioeconomic status (ß= 0.474, p=0.001) was the only factor associated with the full-scale IQ whereas CNS involvement (ß=−0.074, p=0.62), duration of hospital stay (ß=−0.257, p=0.08) and eGFR at time of discharge (ß=−0.117, p=0.41) were not.

Discussion

The majority of follow-up studies of children with HUS have focused on renal outcome after HUS episode [2–5, 9, 15]. Data on neurodevelopmental outcome, however, are scarce, with only few published studies of various designs and case series available [18, 30], and little information on long-term

Table 6 Neurodevelopmental outcome of 38 children with STEC-HUS

Assessment tool	Neurodevelopmental outcome		
	Score	p value	<P10[a]
Wechsler Intelligence Scale 4th version			
Full-scale IQ	104.5 (82–127)	0.003*	
Verbal comprehension index	104 (79–124)	0.10	
Perceptional reasoning index	108 (86–123)	0.001*	
Working memory index	102 (82–135)	0.06	
Processing speed index	100 (79–129)	0.25	
Zurich Neuromotor Assessment			
Timed performances			
Pure motor	0.1 (−2.4 to 4.6)	0.91	11 % (4/38)
Adaptive fine motor	−0.3 (−3.6 to 3.5)	0.18	24 % (9/38)
Adaptive gross motor	−0.8 (−7.5 to 2.5)	0.012*	34 % (13/38)
Static balance	−0.2 (−2.9 to 1.7)	0.13	11 % (4/38)
Associated movements	–1.2 (−3.2 to 2.0)	<0.001*	39 % (15/38)

*Significant difference at $p< 0.05$

Results are presented as the median score with the range given in parenthesis. Comparison is to test norms (mean IQ 100, standard deviation 15)

STEC-HUS, *Escherichia coli* hemolytic uremic syndrome

[a] <P10 indicates the proportion of patients presenting with z-scores of< −1.282 (i.e. results<10th percentile)

outcome. We report here our results from a single-center cross-sectional investigation assessing neurocognitive and neuromotor long-term outcome of pediatric patients after STEC-HUS, P-HUS or aHUS. In our study we also examined the role of CNS involvement during the acute episode of HUS on subsequent neurodevelopment. In contrast to previous studies focusing on STEC-HUS [17–19], we expressly included patients with different HUS forms (STEC-HUS, P-HUS and aHUS) even though apart from thrombotic microangiopathy the underlying pathomechanisms of these HUS forms do differ.

Our patient series showed an overall favorable neurodevelopmental outcome after a history of HUS, with a normal full-scale IQ. Furthermore, the intellectual performance of our patients was not affected by CNS impairment during the acute HUS episode. Only socioeconomic status was positively correlated with full-scale IQ, which is consistent with findings in healthy controls [26]. Socioeconomic status is also a strong predictor of intellectual outcome in other populations at risk, such as preterm born children [31] or children with congenital heart defects [32].

One-third of our study patients presented with neurological symptoms during the acute episode of HUS, particularly in the form of seizures and altered consciousness, including four of the six patients with P-HUS, but none of those with aHUS. These findings are consistent with those of previous studies on neurological involvement in patients with STEC-HUS (19–30 % showing neurological symptoms) [2, 9, 33] and P-HUS patients (16–56 % with neurological symptoms) [14, 34, 35]. There are no evidence-based guidelines on the treatment of CNS complications in HUS. Nathanson et al. [16] suggested that plasmapheresis might have some benefit in children with severe CNS complications. In our series, only two children with STEC-HUS underwent plasmapheresis for severe neurological complications with full neurological recovery.

Our findings are consistent with the results of three previous studies. Schlieper et al. [17] demonstrated a favorable neurocognitive outcome in children at a mean age of 8.6 years (±3.1 SD) and mean duration of 4.1 years (±2.4 SD) after the diagnosis of HUS, with normal full-scale and subscale IQ values in 91 children after HUS (without specification of HUS type), including nine children with seizures or coma during the acute episode of HUS. However, these authors did observed mild deficits in language domains in patients with severe acute HUS [17]. Qamar et al. [18] described a normal intellectual outcome in all seven patients studied despite severe neurological complications during the acute disease. Bauer et al. [19] also reported a favorable neurocognitive outcome in 25 children affected by the STEC-HUS outbreak in 2011 in Germany due to *E. coli* O104:H4. However, these authors observed a slightly lower full-scale IQ in children with CNS involvement vs. those without CNS involvement during HUS. Other studies focusing on neurological involvement in adult patients with STEC-HUS due to *E. coli* O104:H4 also suggested a good neurological outcome [36].

In our series, only six children (12 %), including four with ESRD, had a full-scale IQ of <85, indicating an unfavorable intellectual outcome. Moreover, two of these six children had a history of severe cerebral complications after P-HUS.

Patients with a history of ESRD showed a significant poorer neurocognitive outcome after HUS compared to patients without ESRD after HUS. The development of ESRD, particularly in infancy, is a known risk factor for impaired neurocognitive outcome [37].

In our study, neuromotor performance was less favorable than intellectual outcome, with a poorer outcome particularly in fine and gross motor functioning, static balance and movement quality. Normal performance observed in the domain of pure motor functioning. This is an interesting finding. In this domain, simple motor tasks, such as repetitive or sequential finger, hand or foot movements, are performed. It is conceivable that impairments only become apparent when more complex motor functions, such as adaptive motor performances, are required. Motor performance did not differ between children with and without CNS impairment during acute HUS episode. Qamar et al. [18] also studied neuromotor outcome after HUS, reporting impaired fine motor and clumsiness in four of seven patients with severe neurological complications during the acute HUS episode. In our study, a significant proportion of patients (15–38 %) had motor performance below the 10th percentile. This poorer motor performance is clinically significant as children who perform below the 10th percentile often have difficulties participating in activities of daily life and demonstrate poorer hand writing skills and slower speed. However, we did not assess the impact of motor difficulties on daily life. Of note, motor therapies were reported in 19 % of our patients independently of CNS involvement during HUS.

The pathophysiological mechanisms leading to impaired neuromotor outcome after HUS remain to be clarified. In addition to cerebral thrombotic microangiopathy, factors such as electrolyte imbalances (e.g. severe hyponatremia), hypoosmolality, azotemia, arterial hypertension and the direct toxic effects of Shiga toxin in STEC-HUS may be involved in pathogenetic mechanisms of neurological impairment [19, 38]. Impaired neuromotor outcome can also be found in other cohorts of pediatric patients with various diseases, such as congenital diaphragmatic hernia [39] or congenital heart disease [40]. Impaired neuromotor findings also suggest disease-unrelated factors leading to an adverse neuromotor performance, such as factors attributable to long hospital stay secondary to more severe course of a disease or more parental protection and less experience.

This study has several limitations. Due to its retrospective and cross-sectional study design, information on neurological complications during the acute illness phase was obtained

retrospectively by chart review and parental interviews. Apart from the parents' medical report, no formalized data on the neurodevelopmental status prior to HUS were available. Furthermore, the number of patients with P-HUS and aHUS was too small to analyze outcome in relation to HUS type.

In conclusion, the results of this study show that children and adolescents with HUS have a normal intellectual outcome, but a significant impairment in motor outcome. Neurological complications during the acute episode of HUS were not associated with a poorer neurodevelopmental outcome. Therefore, long-term observation of children after HUS is advisable for the early detection of neurodevelopmental deficits.

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Conflict of interest None.

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ORIGINAL ARTICLE

Long-term health-related quality of life and psychological adjustment in children after haemolytic-uraemic syndrome

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Abstract
Background In children after haemolytic-uraemic syndrome (HUS), little is known about long-term health-related quality of life (HRQoL) and psychological adjustment as defined by behavioural problems, depressive symptoms and post-traumatic stress symptoms.
Methods Sixty-two paediatric patients with a history of HUS were included in this study. Medical data of the acute HUS episode were retrieved retrospectively from hospital records. Data on the clinical course at study investigation were assessed by clinical examination and laboratory evaluation. HRQoL and psychological adjustment data were measured by standardised, parent- and self-reported questionnaires.
Results Haemolytic-uraemic syndrome was diagnosed at a mean of 6.5 years before the initiation of the study (standard deviation 2.9, range 0.1–15.7) years. Among the preschool children, parents reported that their child was less lively and energetic (HRQoL emotional dimension), while no increased behavioural problems were reported. In the school-age children, self- and proxy-reported HRQoL was well within or even above the norms, while increased total behavioural problems were found. The school-age children reported no increased depression scores. Also none of the children met the criteria for full or partial HUS-associated posttraumatic stress disorder.
Conclusions Healthcare providers should be particularly alert to behavioural problems in school-age children with a history of HUS and to lower HRQoL in preschool children.

Keywords Kidney disease · Outcome · Behavioural problems · Depression · Post-traumatic stress disorders · Paediatric patients

Introduction

Haemolytic-uraemic syndrome (HUS) is a systemic and life-threatening disease, and one of the main causes of acute kidney injury in children [1]. Most paediatric patients suffer from typical, infection-mediated forms of HUS [i.e. shigatoxin-associated HUS (STEC-HUS) or *Streptococcus pneumoniae*-associated HUS (P-HUS)] [2–5]. In the acute phase of the disease, many patients need treatment in an intensive care unit (ICU) and dialysis. While renal function recovers in the majority of HUS patients, a few patients develop chronic kidney disease (CKD), leading to end-stage renal disease (ESRD) [3, 5, 6]. Thus, HUS patients are at risk of late and long-term renal and extra-renal complications, such as neurological sequelae, visual disorders and diabetes mellitus [2], that may be very stressful and impact the patients' health-related quality of life (HRQoL) and psychological adjustment.

HRQoL and psychological adjustment have become two important outcome measures to evaluate the impact of a

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disease on an individual patient. While HRQoL is a multidimensional concept that focuses on the subjective perception of physical, emotional, social and cognitive dimensions of health [7], psychological adjustment targets the individual's mental health by asking about the presence or absence of behavioural problems and/or psychological symptoms (e.g. depression) [8]. In contrast to the well-described medical outcome of HUS [2–6, 9], little is known about the psychological outcome and HRQoL in children after HUS. The few studies which have been conducted [9–11] indicated good psychological adjustment as measured by child behavioral problems 4 years after the acute HUS episode, but none of these studies examined the child's HRQoL. In recent years, HRQoL has been mainly studied in paediatric patients with CKD, including kidney transplant recipients, with the results indicating that their HRQoL was lower than that of healthy controls [12–19]. Research on determinants of HRQoL has revealed that transplant recipients have better HRQoL than dialysis patients [16, 19], while the results for differences in HRQoL between patients on conservative treatment and dialysis are inconsistent [12–14]. Higher proportions of behavioural and emotional disorders among paediatric patients with CKD have also been shown [15, 20–24]. However, none of these studies with CKD patients, as well as with children with a history of HUS, examined posttraumatic stress symptoms. Since HUS is a life-threatening event, children may be traumatised and subsequently develop posttraumatic stress symptoms or even posttraumatic stress disorder (PTSD), as based on the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) [25].

The aims of the study reported here were threefold. Firstly, we wanted to examine self- and proxy-reported HRQoL in children with a history of typical and atypical HUS forms, as compared to healthy controls. A lower HRQoL was expected in long-term survivors of HUS as these children are at risk of severe complications. Secondly, we wanted to describe the psychological adjustment of these children by examining parent-reported behavioural problems, self-reported depressive symptoms and PTSD symptoms in school-age children only. We assumed that behavioural problems were more common in these children than in age-matched healthy controls and that a small proportion of school-age children would report depressive symptoms and PTSD symptoms secondary to HUS. This was also hypothesized based on the fact that HUS patients are at risk of late and long-term renal and extra-renal complications which might negatively impact the patients' psychological adjustment. Thirdly, we aimed to examine associations between children's medical characteristics and long-term HRQoL and psychological adjustment. We expected to identify lower HRQoL and lower psychological adjustment in patients with current lower renal function.

Methods

Participants and procedure

This cross-sectional study was part of a comprehensive single-centre data collection process on long-term renal, neurodevelopmental and psychosocial outcomes in paediatric patients with a history of HUS [26, 27]. The study was approved by the Ethics Committee and registered at ClinicalTrials.gov (NCT 01666548). For this study, children and adolescents with a diagnosis of HUS based on current HUS nomenclature [28] and treated at the Paediatric Nephrology Unit of the University Children's Hospital Zurich between April 1995 and February 2013 were recruited between February 2012 to February 2013. Children with severe mental retardation (e.g. with trisomy 21) before the episode of HUS and children aged <18 months or >17 years at the time of assessment were excluded from this study.

Overall, 107 children with a HUS history were eligible for study participation. Of these, 45 children did not participate for the following reasons: child deceased before inclusion ($n = 7$), insufficient knowledge of German ($n = 1$), lost to follow-up ($n = 16$), siblings ($n = 3$; both siblings took part in the study, but only 1 child was included in the analysis because parent-reported data on HRQoL and psychological adjustment are correlated in these cases) and no reasons given ($n = 18$). Ultimately, 62 children participated in this study (response rate 58%). All parents of these children signed the informed consent form. The 62 participants did not differ from the 45 non-participants in terms of sex ($\chi^2 = 0.10$, $p = 0.84$), age at HUS diagnosis ($U = -0.98$, $p = 0.33$), type of HUS classification ($\chi^2 = 0.75$, $p = 0.45$), need of dialysis during acute HUS ($\chi^2 = 0.09$, $p = 0.84$), length of hospital stay during acute HUS ($U = -0.21$, $p = 0.84$), development of CKD at study investigation ($\chi^2 = 0.43$, $p = 0.54$) and socioeconomic status (SES; $U = -0.31$, $p = 0.76$).

After receiving written informed consent, the child's medical data on the acute HUS episode were retrieved retrospectively from the patient's hospital records. Upon inclusion in the investigation, all children underwent clinical examination and laboratory evaluation. All parents were asked to complete standardised questionnaires on their child's HRQoL and psychological adjustment. Self-reported HRQoL and psychological adjustment of children aged >6.5 years were obtained by standardised questionnaires filled in by an experienced paediatrician in a face-to-face interview with the patient. An

Table 1 Overview of long-term outcome measures assessed in the study

	Preschool children aged ≤ 6.5 years		School-age children aged >6.5 years		
	Outcome measure	Proxy-reported	Outcome measure	Self-reported	Proxy-reported
Health-related quality of life (HRQoL)	TNO-AZL Preschool Quality of Life Questionnaire (TAPQOL)	x	Health-related quality of Life Questionnaire (KIDSCREEN)	x	x
Psychological adjustment					
Behavioural problems	Child Behaviour Check List (CBCL) for ages 1.5–5 and 4–18 years	x	CBCL for ages 4–18 years	-	x
Depression	-	-	Child Depression Inventory (CDI)	-	x
Posttraumatic stress disorder	-	-	University of California at Los Angeles (UCLA) PTSD Reaction Index	-	x

PTSD, Posttraumatic stress disorder

overview of all outcome measures assessed in the study can be found in Table 1.

Measures

Medical data

Haemolytic-uraemic syndrome has been defined as the occurrence of non-immune haemolytic anemia, thrombocytopenia and features of acute renal injury [26]. The diagnosis of HUS was confirmed in all patients by paediatric nephrologists and classified as (1) typical, infection-mediated HUS, including STEC-HUS and P-HUS, or as (2) atypical HUS (aHUS) based on hereditary and/or acquired disorders of regulation of the alternative complement system. Neurological involvement during acute HUS included seizures, altered consciousness, ataxia, muscle tone abnormalities, hemiplegic symptoms, dysarthria, visual disorders, movement disorders and vestibular symptoms [26]. Long-term renal sequelae included the presence of CKD, as defined according to the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation [29]. Glomerular filtration rate as a marker of renal function and its classification into the five CKD stages was evaluated using the Schwartz formula with the local factor *k* of 40 for all children and with plasma creatinine concentration in micromoles per litre [30]. The time since acute HUS was calculated by the difference between the child's age at study investigation and age at HUS diagnosis.

Health-related quality of life

Health-related quality of life for children aged ≤6.5 years was assessed by parental report, using an authorised German version of the TNO-AZL Preschool Quality of Life Questionnaire (TAPQOL) [31], and for participants aged >6.5 years of age using the German parent and child form of the KIDSCREEN-27 [32].

The TAPQOL is a validated and standardised questionnaire consisting of 43 items with a recall period of 1 week. The items are classified into 12 scales assessing four global dimensions of HRQoL: physical functioning (including information on the child's sleeping and appetite problems), social functioning (measuring social contacts with other children, such as whether the child was at ease with other children), cognitive functioning (measuring the communicative skills of the child) and emotional functioning (measuring the child's level of anxiety, positive mood and liveliness). Scales are transformed into a 0–100 scale, and the four global dimensions are computed by the average of all underlying scales. To obtain a measure of overall HRQoL, the total score is computed by averaging the scores of the four global dimensions. Higher scores indicate better HRQoL. We retrieved norms from the manual, and these were based on data from 211 parents of healthy Dutch children between the ages of 18 months and 5 years [31]. In this study, internal consistency was good for the total score (Cronbach's $\alpha = 0.80$) and for most scales, except for the scales measuring lung problems (Cronbach's $\alpha = -0.14$) and social functioning (Cronbach's $\alpha = -0.08$), which displayed poor internal consistencies.

The KIDSCREEN-27 is a validated and standardised questionnaire assessing self- and proxy reports of HRQoL in children ranging in age from 8 to 18 years [33]. It consists of 27 items assessing either the frequency or the intensity of a behaviour or a feeling on a 5-point Likert scale, over a recall period of 1 week. The following five HRQoL dimensions are computed in KIDSCREEN-27: physical well-being, psychological well-being, autonomy and parent relations, peers and social support and school environment. In accordance with the manual, scale scores are transformed into T-values based on international reference data [32]. In our study, T-values were compared to data from a community sample of >1596 Swiss

children between the ages of 8 to 18 years. A total score is computed from the average of the T-values of the five HRQoL domains. Internal consistency in this study was good for the total score (Cronbach's $\alpha = 0.87$ for both parent- and self-reported total score) and acceptable to good for the five HRQoL domain scales (Cronbach's ranging from $\alpha = 0.68$ to $\alpha = 0.84$ for parent-reported HRQoL scores and from $\alpha = 0.61$ to 0.72 for self-reported HRQoL scores).

Psychological adjustment

The Child Behaviour Checklist (CBCL) is a validated and standardised questionnaire assessing parental reports of a child's behavioural problems [34, 35]. In this study, two authorised German versions of the CBCL were used: the CBCL/1.5-5 for children aged <4 years [36] and the CBCL/4-18 for children aged ≥4 years [34]. Both instruments yield scores for internalising behavioural problems (referring to behaviours in which children direct feelings and emotions inwards, such as being anxious or depressed, having somatic complaints or social withdrawal), externalising behavioural problems (referring to behaviours which are directed outward, such as being aggressive or delinquent) and an overall score for total behavioural problems. Higher scores indicate more behavioural problems, or less psychological adjustment. T-values are derived from normative data: the T-values of the CBCL/1.5-5 were calculated based on a community sample of 700 healthy U.S. children [35], the T-values of the CBCL/4-18 were calculated based on 1964 healthy Swiss children [37]. In the current study, internal consistency for the CBCL/1.5–5 or CBCL/4-18 was acceptable to good for internalising problems (Cronbach's $\alpha = 0.82$, and $\alpha = 0.73$, respectively), externalising problems (Cronbach's $\alpha = 0.79$, and $\alpha = 0.90$, respectively) and total problems (Cronbach's $\alpha = 0.87$, and $\alpha = 0.88$, respectively).

The presence and severity of self-reported depressive symptoms of school-age children only were assessed using the authorised German version of the Child Depression Inventory [38], i.e., the "Depressions-Inventar für Kinder und Jugendliche" (DIKJ) [39]. The DIKJ is validated for children between 8 and 17 years of age and includes 26 items with three response options for each item, representing the severity of depression ranging from 0 (no symptoms) to 2 (definite symptoms). A total score is obtained by summing across all 26 items. Higher values indicate greater depression severity. Clinically relevant depression was defined by a cut-off score set at total score of >18 [39]. In the current study, internal consistency for the total score was acceptable (Cronbach's $\alpha = 0.69$).

PTSD in the school-age children was assessed using the German child version of the University of California at Los Angeles (UCLA) PTSD Reaction Index [40] which is validated for children aged >6 years [41]. The UCLA PTSD provides a diagnosis of PTSD according to the criteria of the DSM-IV [25]. The first part of the UCLA PTSD assesses the type of traumatic event (e.g. having had a life-threatening disease like HUS) and, in case there were multiple events, the most stressful one. The second part evaluates the circumstances and the emotions in the context of the trauma. The third part assesses the frequency of the 20 PTSD symptoms on a 5-point Likert scale (0–4) with regard to the last month. Symptoms are related to the three DSM-IV clusters (i.e. intrusion, avoidance and hyperarousal). Higher scores indicate greater symptom severity. The fourth part of the questionnaire assesses the duration of symptoms and possible functional impairments in different areas of life (e.g. school and learning). According to the DSM-IV, criteria for full PTSD are met if the child reports at least one intrusion symptom, three avoidance symptoms and two hyperarousal symptoms, as well as duration of these symptoms for at least 1 month and impaired function in at least one life area [25].

Socioeconomic status

Socioeconomic status was assessed on the basis of maternal education and paternal occupation, ranging from 2 to 12, with 2 being the lowest SES score and 12 the highest. Three social classes were assigned: lower (SES 2–5), middle (SES 6–9) and upper (SES 10–12). This measure has proven to be a valid indicator of SES in previous studies involving the Swiss population [42].

Statistical analyses

Data were analysed using the statistical package SPSS for Windows, release 22 (IBM Corp., Armonk, NY). All analyses were performed with two-tailed tests, and $p < 0.05$ was considered to be significant. Chi-square tests and Mann–Whitney *U* tests were used as appropriate to compare child sex, child age at HUS diagnosis, type of HUS classifications, need for dialysis during the acute phase, length of hospital stay during acute HUS, development of CKD and SES between participants and non-participants. Cronbach's alpha was calculated to test the internal reliability of scale scores. Because most data showed non-normal distributions according to the Kolmogorov–Smirnov test, we used Mann–Whitney *U* tests for testing the equality of means between preschool and school-age children. Differences in child HRQoL and psychological adjustment scores between sample means and normative data were examined using Mann–Whitney *U* tests or one-sample *t* tests. These differences were quantified by calculating effect sizes according to Cohen with a *d* of 0.20 indicating a small effect, a *d* of 0.50 indicating a medium-sized effect and a *d* of > 0.80 indicating a large effect [43]. Pearson correlation was calculated to analyse associations between

sociodemographic variables, medical characteristics, HRQoL and psychological adjustment scores.

Results

Patient characteristics

Patient characteristics are summarised in Table 2. The majority of the children had a STEC-HUS, while seven children also had a P-HUS and three school-age children had aHUS. At the time of HUS diagnosis, the children were on average 2.9 years old [standard deviation (SD) 2.9, range 0.3–14.4) years. During the acute episode, 43 of the 62 children (69%) were dialysed; in most cases, dialysis consisted of peritoneal dialysis. All but five children (92%) were initially treated in the ICU, with school-age children more frequently treated in ICU than preschool children. On average, the children stayed for 24 days in hospital. Neurological involvement was present in 24 of the 62 children (39%).

The average age of the children at the time of the study investigation was 9.4 (SD 4.1, range 1.9–16.7) years. Of the 62 children enrolled in the study 26 (42%) had CKD, among whom 11 had CKD stage 1, nine had stage 2, one had stage 3 and five had ESRD (CKD stage 5). Four children with ESRD (STEC-HUS, $n = 2$; P-HUS, $n = 1$; aHUS, $n = 1$) had undergone successful renal transplantation, while one child with STEC-HUS was still on the waiting list for renal transplantation. In addition, four of the 26 patients with CKD (15%) had severe long-term extra-renal sequelae. One patient had insulin-dependent diabetes mellitus due to STEC-HUS-related pancreatitis, one patient had impaired visual acuity due to retinal bleeding during acute STEC-HUS and two patients had severe neurodevelopmental impairment due to severe neurological involvement in STEC-HUS and in P-HUS following pneumococcal meningitis, respectively. Four children with normal renal function had been diagnosed with another chronic disease since their HUS diagnosis, i.e. attention deficit hyperactivity disorder, epilepsy, hearing disorder and neurodevelopmental delay. Thus, 32 of the 62 children (52%) had neither renal nor extra-renal sequelae.

Health-related quality of life

Mean values of HRQoL scores for the study sample and the reference group are listed in Tables 3 and 4 for preschool children and school-age children, respectively. Parents of preschool children did not report impaired overall HRQoL as measured by the TAPQOL. Indeed, they described their child as having better social function than the reference group. However, the parents did report that their preschool-age child had been less lively and energetic (emotional HRQoL dimension). In contrast, parents of school-age children reported increased overall HRQoL as well as better psychological well-being and more supportive interactions with their peers than the control group. School-age children (child self-report form) reported all HRQoL dimensions within norms.

Psychological adjustment

Sample means and reference data for child behavioural problems are listed in Table 5. Parents of preschool children did not report that their child had more total behavioural problems than controls. They even reported significantly fewer externalising problems. In contrast, parents of school-age children with a HUS history reported significantly more total behavioural problems than parents of control children. Clinically relevant internalising problems were reported for one preschool child (10%) and four school-age children (9%); externalising problems above the cut-off for clinically relevant problems were reported for three school-age children only (7%).

Data on self-reported depressive and PTSD symptoms were available for 37 of the 41 school-age children. The average depression score was 8.5 (SD 5.0, range 0–28). A total depression score above the cut-off value for clinically relevant depression was reported for only one patient (3%), a girl with STEC-HUS and currently normal renal function. In addition, none of the 37 school-age children perceived HUS as a traumatic event and met the criteria for full or partial PTSD according to DSM-IV.

Associations between medical data, HRQoL and psychological adjustment

Bivariate correlations between the child's socio-demographic characteristics (age, sex, SES), medical characteristics, long-term HRQoL and psychological adjustment scores are presented in Table 6. Among the preschool children, no significant associations were found between medical characteristics and HRQoL or psychological adjustment scores, while parents from lower SES classes exhibited significantly more behavioural problems. Among the school-age children, children with CKD at study investigation self-reported that they had lower HRQoL than children without CKD. This effect was not found for parent-reported HRQoL. In addition, longer periods in ICU, length of dialysis and length of periods in hospital were related to more total behavioural problems in the school-age children. These medical characteristics were not significantly associated with self-reported depressive symptoms, while a shorter time since HUS diagnosis was associated with more depressive symptoms.

Discussion

We have investigated both HRQoL and psychological adjustment in children with a history of HUS and the associations between these two outcome measures and the child's medical characteristics. Our results indicate that, on average 6.5 years after acute HUS, self- and proxy-reported HRQoL of school-age children was well within or even above the norms, while some psychological maladjustment, as measured by the CBCL, was present. Among the preschool children, parents reported that their child was less lively and energetic (emotional HRQoL dimension) while no increase was found in behavioural problems.

In contrast to our hypothesis, overall HRQoL of the children with a history of HUS was not impaired. As none of the previous studies with paediatric patients with a history of HUS examined HRQoL, we were not able to compare this result with any data reported in the literature. Our result that parents

Table 2 Characteristics of paedriatic patients with haemolytic-uraemic syndrome enrolled in this study

Patient characteristics[a]	Total sample ($n = 62$)	Preschool children ($n = 21$)	School-age children ($n = 41$)	p[b]
Age at investigation (years)	9.4 (4.1) [1.9–16.7]	4.7 (1.4) [1.9–6.5]	11.8 (2.7) [6.75–16.7]	<0.001
Female gender	35 (57%)	12 (57%)	23 (56%)	0.94
Type of HUS classification				
STEC-HUS	52 (84%)	18 (86%)	34 (83%)	0.20
P-HUS	7 (11%)	3 (14%)	4 (10%)	
aHUS	3 (5%)	0	3 (7%)	
Age at diagnosis (years)	2.9 (2.9) [0.3–14.4]	1.9 (1.0) [0.3–4.1]	3.4 (3.4) [0.4–14.4]	0.28
Time since acute HUS (years)	6.5 (4.1) [0.1–15.7]	2.8 (1.7) [0.1–6.1]	8.4 (3.6) [0.3–15.7]	<0.001
Socioeconomic status	8.4 (2.1) [2–12]	8.1 (1.8) [4-12]	8.5 (2.3) [2–12]	0.39
Medical characteristics during acute episode of HUS				
Dialysis	43 (69%)	13 (62%)	30 (68%)	0.36
Peritoneal dialysis	32 (74%)	9 (69%)	23 (77%)	
Haemofiltration/haemodialysis	8 (19%)	4 (31%)	4 (13%)	
Combination of peritoneal and haemofiltration/haemodialysis	3 (7%)	0	3 (10%)	
Duration of dialysis (days)	10.3 (14.3) [0–79]	10.0 (16.9) [0–78]	10.5 (13.1) [0–79]	0.38
Stay in ICU	57 (92%)	17 (81%)	40 (98%)	0.02
Duration of stay in ICU (days)	8.2 (7.3) [0–31]	7.9 (7.3) [0–22]	8.3 (7.4) [0–31]	0.75
Duration of hospital stay (days)	23.7 (17.7) [5–97]	21.7 (18.7) [5–92]	24.8 (17.3) [5–97]	0.33
Neurological involvement	24 (39%)	9 (43%)	15 (37%)	0.63
Clinical course at investigation				
CKD	26 (42%)	7 (33%)	19 (46%)	0.33
Stage 1	11 (42%)	5 (36%)	6 (32%)	
Stage 2	9 (35%)	1 (7%)	8 (42%)	
Stage 3	1 (4%)	0	1 (5%)	
Stage 4	0	0	0	
Stage 5	5 (19%)	1 (7%)	4 (21%)	
CKD and other long-term extra-renal sequelae	4 (15%)	2 (29%)	2 (11%)	0.48
Not HUS-related comorbidities	4 (7%)	1 (5%)	3 (7%)	0.70

HUS, Haemolytic-uraemic syndrome; STEC-HUS, shigatoxin-associated HUS; P-HUS, *Streptococcus pneumoniae*-associated HUS; aHUS, atypical HUS; CKD, chronic kidney disease; ICU, intensive care unit

[a] Values in table are presented as a number with the percentage in parenthesis or as the mean with the standard deviation (SD) in parenthesis and the range in square brackets

[b] Mann–Whitney U tests were performed for continuous variables and χ^2 tests were performed for nominal variables (all dichotomised)

Table 3 Sample means and reference data for health-related quality of life in preschool children with a history of haemolytic-uraemic syndrome

Measure	Sample			Reference group [a]		Statistics	
	n	Mean	SD	Mean	SD	p^b	d^c
TAPQOL parent form							
Physical functioning							
Sleeping	21	83.1	16.6	84.3	15.7	0.79	−0.07
Appetite	20	85.8	23.4	85.2	11.9	0.06	0.03
Lung problems	20	96.3	8.8	97.7	7.8	0.18	−0.17
Stomach problems	18	86.1	17.2	92.4	13.2	0.08	−0.41
Skin problems	21	88.5	15.7	92.6	10.4	0.55	−0.31
Motor functioning	20	94.1	22.5	98.8	3.7	0.84	−0.29
Physical dimension score	16	90.8	6.4	91.9	5.6	0.51	−0.18
Social functioning							
Social functioning	20	95.8	10.6	91.4	15.1	0.13	0.34
Problem behavior	21	79.8	19.2	66.0	15.1	0.001	0.80
Social dimension score	20	87.5	9.6	78.7	10.4	0.001	0.88
Cognitive functioning							
Communication	20	92.8	15.4	91.8	9.8	0.15	0.07
Emotional functioning							
Anxiety	20	83.3	21.6	78.6	17.9	0.18	0.24
Positive mood	20	97.5	8.2	99.0	5.7	0.20	−0.21
Liveliness	20	90.0	18.3	97.8	8.3	0.002	−0.55
Emotional dimension score	20	90.3	12.3	91.8	7.4	0.53	−0.14
Total HRQoL score	16	86.4	10.5	82.9	7.4	0.09	0.38

Higher scores indicate better HRQoL

[a] Reference group according to Bisegger et al. [32]

[b] One sample *t* tests were performed

[c] Effect size according to Cohen [43]

rated their child's HRQoL as similar to or even better than the norms might be explained by the possibility that they rated their child's current HRQoL in comparison to that of when their child was sick. Our result is indeed in contrast to those of several studies indicating lower HRQoL in paediatric patients with CKD compared to healthy controls [12–19]. In our study, 42% of the children had CKD, among whom four had already undergone renal transplantation and one child was still on the waiting list for renal transplantation. This lower rate of CKD patients might explain the better HRQoL reports in our study. However, our results are in line with a study that showed no difference in long-term HRQoL in children 3–5 years of age after acute renal failure of various causes (e.g. due to nephrotoxicity) [44]. Thus, children with a history of HUS, even

Table 4 Sample means and reference data for health-related quality of life in school-age children with a history of haemolytic-uraemic syndrome

Measure	Sample			Reference group [a]			
	n	Mean	SD	Mean	SD	p^b	d^c
KIDSCREEN-27 parent proxy-report form							
Physical well-being	34	54.6	10.8	52.8	8.7	0.34	0.18
Psychological well-being	34	56.3	10.6	51.9	9.0	0.022	0.45
Autonomy & parents	34	54.9	8.3	53.1	8.4	0.22	0.22
Peers & social support	34	55.1	8.0	51.0	8.1	0.005	0.51
School environment	33	54.8	8.6	52.5	8.9	0.14	0.26
Total score	33	55.3	6.2	52.0	9.0	0.005	0.43
KIDSCREEN-27 child self-report form							
Physical well-being	37	50.4	7.7	52.7	9.0	0.07	−0.28
Psychological well-being	37	53.3	7.2	53.1	9.3	0.86	0.02
Autonomy & parents	37	52.7	8.5	53.4	8.8	0.64	−0.08
Peers & social support	37	52.1	7.9	51.0	9.0	0.40	0.13
School environment	37	54.9	7.0	53.0	9.0	0.11	0.24
Total score	37	52.7	5.4	52.8	9.2	0.90	−0.01

Higher scores indicate better HRQoL

[a] Reference group according to Bisegger et al. [32]

[b] One-sample *t* tests were performed

[c] Effect size according to Cohen [43]

Table 5 Sample means and reference data for behavioural problems of children with a history of haemolytic-uraemic syndrome

Measure	Sample			Reference group[a]			
	n	Mean	SD	Mean	SD	*p*[b]	*d*[c]
CBCL 1.5-5 parent proxy-report form							
Internalising score	10	48.2	14.8	50.0	10.0	0.71	−0.15
Externalising score	9	41.3	6.3	50.0	10.0	<0.01	−1.04
Total score	11	43.8	10.2	50.0	10.0	0.07	−0.61
CBCL 4-18 parent proxy-report form							
Internalising score	45	48.4	10.4	50.0	10.0	0.32	−0.16
Externalising score	46	47.4	9.9	50.0	10.0	0.09	−0.26
Total score	46	55.4	6.5	50.0	10.0	<0.001	0.64

[a] Reference group of the CBCL 1.5-5 according to Achenbach and Rescoria [35] and of the CBCL 4-18 according to Steinhausen et al. [37]

[b] One-sample *t* tests were performed

[c] Effect size according to Cohen [43]

severe cases of HUS, might recover quite well in the long-term, and a patient's acute medical characteristics (e.g. longer length of stay in ICU in severe cases) might have a minor impact on long-term HRQoL. Our data indicate that neither medical characteristics during an acute episode nor the presence of CKD were significantly associated with impaired HRQoL reported by the parents. In contrast, our study showed that lower self-reported HRQoL among the school-age children was associated with the presence of CKD.

With regard to psychological adjustment, the parents of our study cohort reported increased total behavioural problems for school-age children, but not for preschool children, with the parental reports for the latter group even indicating significantly fewer externalising problems. This is in contrast to previous studies which found no clinically significant total behavioural problems in children with a history of HUS [9–11]. Possible factors to explain these differences include the longer time period since acute HUS in the CKD patients and the increased number of children affected by CKD. Thus, especially school-age children with a history of HUS seem to be at risk of psychological maladjustment as measured by the CBCL. Among these children, a longer stay in the ICU as well as longer periods of dialysis and hospital stay were significantly associated with more parent-reported total behavioural problems in our study. Thus, these medical characteristics can be seen as risk factors for long-term psychological maladjustment in children with a history of HUS. Among the school-age children, only one female child with a history of STEC-HUS reported clinically relevant depressive symptoms. However, we cannot prove a direct association with the HUS episode. In fact, none of the school-age children perceived

Table 6 Correlations between child socio-demographic characteristics, medical characteristics, health-related quality of life and psychological adjustment scores

Patient characteristics	Preschool children		School-age children			
	HRQoL	Psychological adjustment	HRQoL		Psychological adjustment	
	Parent-reported TAPQOL total score (*n* = 16)	Parent-reported CBCL total score (*n* = 11)	Parent-reported KIDSCREEN total score (*n* = 33)	Self-reported KIDSCREEN total score (*n* = 37)	Parent-reported CBCL total score (*n* = 36)	Self-reported CDI total score (*n* = 37)
Age at study investigation	−0.30	0.41	−0.18	−0.03	−0.19	0.10
Sex	−0.15	−0.01	0.08	−0.14	−0.13	0.17
Socioeconomic status	0.15	−0.72*	−0.36*	0.12	0.05	−0.06
Type of HUS classification	NA	NA	−0.10	−0.15	0.19	−0.09
Time since HUS diagnosis	−0.13	0.08	0.15	0.10	−0.21	−0.37*
Length of stay in intensive care unit	−0.18	0.22	−0.21	−0.11	0.33*	−0.03
Length of dialysis	−0.08	0.20	−0.12	−0.05	0.49**	−0.11
Length of hospital stay	−0.06	0.17	−0.13	−0.22	0.56***	−0.14
Neurological involvement	0.26	−0.11	0.01	0.01	0.07	−0.03
CKD at study investigation	0.16	0.07	−0.23	−0.35*	0.14	−0.24

*$p < 0.05$, **$p < 0.01$, ***$p \leq 0.001$

NA, Not applicable (no preschool child was affected by typical HUS)

HUS as a traumatic event and met the criteria for full or partial PTSD according to DSM-IV [25]. This is in contrast to the parents, some of whom met the criteria for full or partial PTSD diagnosis [27].

The strengths of this study include the use of well-validated, multidimensional and standardised questionnaires with reference data, the assessment of self- and proxy-reports and the inclusion of children with a broad spectrum of ages and HUS types. Nevertheless, several limitations merit note. Firstly, the cross-sectional design of our study prevents us from drawing any conclusion about causal relations, and we were not able to describe the course of HRQoL or psychological adjustment over time. Secondly, our study included only a small number of preschool children, with no preschool child being affected by an aHUS. Thus, we were not able to provide data on long-term HRQoL and psychological adjustment separately for patients affected by typical and atypical HUS. Looking on mean differences among the school-age children only, it can be assumed that HRQoL and psychological adjustment might be lower in patients with a history of aHUS (data not shown). Thus, further studies are necessary to analyze HRQoL and psychological adjustment separately for the different HUS forms. However, post-hoc power analysis ($\alpha = 0.05$, two-tailed) using the G*power software [45] indicated that, for each *t* test comparing sample means with normative data, the power to detect a large effect size ($d = 0.80$) was adequate for the TAPQOL (0.87), but just below the recommended 0.80 level for the CBCL 1.5-5 (0.75) [43]. For the school-age group, the power to detect a large or a medium effect size was adequate in each analysis, but not adequate to detect small effect sizes. Thus, our sample sizes provided adequate power at the medium to large effect size level, but not sufficient power at the small effect size level. Thirdly, the appropriateness can be questioned of using Dutch norms for the TAPQOL and U.S. norms for the CBCL 1.5-5. Slight differences with respect to child and parents' age, SES and cultural background may compromise comparability. However, there are no Swiss or German reference values available for these two measures, and previous findings support the use of U.S. norms for the CBCL in German samples [46]. Fourthly, no self-reports of child behavioural problems were assessed, which might have provided an insight into whether the parents underestimated the impact of CKD on their child's psychological adjustment. Fifthly, we were unable to control for confounding factors due to the small sample size for self- and/or proxy-reported HRQoL and psychological adjustment.

We previously recommended that healthcare providers should pay special attention to parents' reports of PTSD symptoms during the clinical follow-up of a child with HUS [27]. Based on the results of our current study, we further recommend that healthcare providers be especially alert for any signs of behavioural problems in school-age children with a history of HUS and for of lower HRQoL in the preschool children.

Compliance with ethical standard

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Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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ORIGINAL ARTICLE

Health-related quality of life and mental health in parents of children with hemolytic uremic syndrome

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Abstract
Background Little is known about health-related quality of life (HRQoL) and mental health of parents having children with a history of hemolytic uremic syndrome (HUS).
Methods This study included 63 mothers and 58 fathers of a cohort of 63 HUS-affected children. At assessment, the mean time since a child experienced an acute episode of HUS was 6.4 years. Parental HRQoL, mental health and posttraumatic stress disorder (PTSD) were assessed with standardized self-report questionnaires. Medical data were extracted from patients' hospital records.
Results The HRQoL and mental health of both the mothers and fathers were not impaired compared to normative data. However, a shorter time since a child's acute HUS episode was a significant predictor of lower HRQoL among the mothers, while no such effect was found among the fathers. Two fathers (3 %), but no mothers, met the criteria for a diagnosis of HUS-related full PTSD; one father (2 %) and four mothers (6 %) met the criteria for a diagnosis of HUS-related partial PTSD.
Conclusions Our study shows that most parents of our study sample were doing well in terms of HRQoL and mental health, although a small number met the criteria for full or partial PTSD diagnosis due to their child's HUS. We therefore recommend that healthcare providers pay special attention to parents regarding PTSD symptoms during the clinical follow-up of a HUS-affected child since some parents may benefit from psychological support.

Keywords Posttraumatic stress disorder · Nephrology · Psychology · Outcome · Pediatric · Parents

Kathrin Buder and Helene Werner contributed equally to this work.

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Introduction

Hemolytic uremic syndrome (HUS) is a systemic, life-threatening disease that usually occurs in previously healthy children and is one of the commonest causes of acute kidney injury in childhood [1]. Most patients are affected by typical, infection-mediated HUS types [i.e. Shigatoxin-associated HUS (STEC-HUS) or *Streptococcus pneumoniae*-associated HUS (P-HUS)] and recover in the majority of cases after appropriate treatment [2–4]. However, about 5–10 % of patients die during the acute phase of the disease while others develop chronic kidney disease (CKD) ranging from preserved kidney damage with normal renal function to end-stage renal disease (ESRD) requiring renal replacement therapy [2–7]. In addition to renal sequelae, HUS may be complicated by long-term extrarenal sequelae, such as neurological impairment, gastrointestinal complications, diabetes mellitus or visual disorders [2–4, 8–10].

For most parents, the experience of watching their child endure a HUS episode combined with the strains of a hospital stay with frequent need of intensive care treatment, the necessity of regular follow-up assessments at the hospital and the

fear of long-term sequelae may be stressful and possibly negatively influence their health-related quality of life (HRQoL). HRQoL is a multidimensional concept integrating the subjective perception of an individual's physical, psychological and social function [11]. To date, no study has described the HRQoL of parents having children with a history of HUS, irrespective of the clinical course of the disease. Most available studies have analyzed parental HRQoL in the context of advanced pediatric CKD, with the results showing that parents of these children have an increased risk of low HRQoL [12–16]. A previous qualitative study of parents of children with a history of STEC-HUS indicated that the parents may also experience increased emotional distress [17]. Thus, it would appear that parents of children with a history of HUS may be at risk of impaired mental health. Additionally, since HUS is an acute life-threatening disease, parents may be traumatized and subsequently develop posttraumatic stress symptoms or even posttraumatic stress disorder (PTSD) based on the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) [18]. PTSD is characterized by psychological reactions following exposure to a traumatic event that are assigned to three symptom clusters (intrusion, avoidance and hyperarousal). A number of studies have reported a high prevalence of PTSD among parents of children with various life-threatening diseases [19–21]. However, no such information is currently available for parents with HUS-affected children.

The aims of the present study were to investigate the HRQoL and mental health of parents having children with HUS as compared to normative data, as well as to assess the prevalence of parental HUS-related PTSD. Knowledge of such impairments might help to identify those aspects of the clinical experience which most strongly affect the parents and, if necessary, to enable these parents to be monitored more closely in future. This, in turn, may help the child to adjust to the disease, as it has been shown that parents with increased problems may be too absorbed in regulating their own feelings to be able to provide sensitive support for their child [22]. Based on the findings of previous studies with parents of children with other suddenly occurring diseases [23, 24] or with advanced stages of CKD [12, 13], we hypothesized that mothers and fathers of HUS-affected children would report a lower HRQoL compared to normative data and that this would be especially evident among the parents of children with atypical HUS (aHUS) and P-HUS, since the course of their disease may be more severe than that of children with typical HUS. Furthermore, taking into account previous investigations of PTSD in parents of children with various life-threatening and/or chronic diseases [19–21], we expected impaired mental health and the presence of HUS-related PTSD symptoms in both the mothers and fathers of HUS-affected children.

Methods

Study population and procedures

Parents whose children were treated at the Pediatric Nephrology Unit of the University Children's Hospital Zurich between April 1995 and February 2013 were recruited for this study which covered the period from February 2012 to February 2013. The inclusion criteria were (1) diagnosis of pediatric HUS based on current HUS nomenclature [25, 26] and (2) child age of <18 years during the 1-year recruiting period. Overall, 115 families with at least one HUS-affected child aged <18 years were treated in our unit during the recruiting period. Thirty-two families were excluded from the study for the following reasons: death of their child during an acute episode of HUS ($n = 7$), loss to follow-up ($n = 18$), insufficient knowledge of German language ($n = 3$) and having more than one HUS-affected child, which differentiates them from all other families, especially with respect to the level of parental stress ($n = 4$). Ultimately, 83 families (100 %) with one HUS-affected child were eligible for the study. The 32 excluded children did not differ significantly from the 83 eligible children with regard to sex, age at HUS diagnosis, need of dialysis during acute HUS, length of hospital stay, development of ESRD or socioeconomic status (SES). Of the 83 families, 20 declined to participate in the study (response rate 76 %). The final sample therefore included 63 mothers and 58 fathers with one HUS-affected child. The 20 children of the non-participating families did not differ significantly from the 63 children of the participating families with regard to sex, age at HUS diagnosis, need of dialysis during acute HUS, length of hospital stay, development of ESRD or SES.

This cross-sectional investigation was part of a comprehensive single-center study of long-term renal, neurodevelopmental and psychosocial outcomes in pediatric patients with a history of HUS. The study was approved by the Ethics Committee of the Canton of Zurich and registered at ClinicalTrials.gov (NCT 01666548). After receiving written informed consent, parents were asked to complete the questionnaires described below. Medical data relating to the acute HUS episode were retrieved retrospectively from the patients' hospital records. For assessment of the clinical course after HUS, all children underwent a clinical examination during the recruiting period. Parental data were also assessed within this period.

Child medical data

Hemolytic uremic syndrome has been defined as the occurrence of non-immune hemolytic anemia, thrombocytopenia and features of acute renal injury [26]. The diagnosis of HUS was confirmed in all patients by pediatric nephrologists and categorized as (1) typical, infection-mediated HUS,

including STEC-HUS and P-HUS, or aHUS based on hereditary and/or acquired disorders of regulation of the alternative complement system.

The Medical data assessed included age at disease onset, requirements, mode and duration of dialysis, length of hospital stay, duration of stay in the intensive care unit during acute HUS, development of ESRD, and the need of chronic renal replacement therapy including dialysis and renal transplantation. CKD was defined according to the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation [7]. Glomerular filtration rate as a marker of renal function and its classification into the five CKD stages was evaluated based on the Schwartz formula using the local factor k of 40 for all children and plasma creatinine concentration expressed in micromoles per liter [27].

Parental HRQoL

Self-reported HRQoL was assessed for the 4 weeks immediately preceding assessment using the authorized German version [28] of the Medical Outcomes Study Short Form-36 item questionnaire (SF-36) [29]. The SF-36 includes eight subscales, namely, physical functions (e.g. difficulty going up stairs), role physical (e.g. difficulties at work due to physical problems), bodily pain (e.g. to what extent pain would restrict everyday activities), general health (e.g. how the general health would be rated compared to the previous year), vitality (e.g. low energy), social functions (e.g. whether physical or emotional problems would influence contact with friends), role emotional (e.g. whether there are problems at work due to mental health problems) and mental health (e.g. being unhappy). The score for each subscale ranges from 0 to 100, with higher scores indicating better HRQoL. The eight subscales can be summarized by two standardized component summary scores, namely,, a physical component summary (PCS) score and a mental component summary (MCS) score, with a mean of 50 [standard deviation (SD) = 10]. In this study, gender-matched German-speaking members of the community ranging in age from 30 to 49 years (mothers) and from 40 to 49 years (fathers), were used as controls to obtain the normative data ($n = 6964$) [30, 31]. These age ranges correspond approximately to the inter-quartile ranges (IQRs) of the study participants' ages. In the study sample, internal consistencies of all subscales were satisfactory to excellent (Cronbach's α between 0.51 and 0.95) and good for the two summary component scores (Cronbach's $\alpha = 0.89$ for the mothers, both PCS and MCS; Cronbach's $\alpha = 0.90$ for the fathers, both PCS and MCS).

Parental mental health

Self-reported mental health was measured with the authorized German version of the Brief Symptom Inventory (BSI) [32, 33]. The BSI includes 53 items regarding the frequency of psychological distress symptoms during the past week. It consists of nine scales: somatization, obsessive–compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism. In addition, the BSI provides an overall measure of psychopathology, i.e., the Global Severity Index. Higher scores indicate lower mental health. Gender-matched T-scores (mean 50, SD 10) were derived based on normative data from a community sample of 600 healthy German adults (300 females and 300 males) [33]. In our study, the internal consistency of the nine scales was found to be satisfactory to good (Cronbach's α between 0.57 and 0.85) and for the Global Severity Index it was excellent (Cronbach's $\alpha = 0.95$ for both mothers and fathers).

Parental PTSD

Posttraumatic stress disorder was assessed by the validated German version of the Posttraumatic Diagnostic Scale (PDS) [34–36], a diagnostic instrument which provides a diagnosis of PTSD according to the criteria of the DSM-IV [18] and a rating of PTSD symptom severity. The PDS consists of four parts. The first part concerns the type of traumatic event and, if there were multiple events, the most stressful one. The second part evaluates the time, the circumstances and the emotions in the context of the trauma. The third part assesses the frequency of 17 PTSD symptoms belonging to the three symptom clusters (i.e. intrusion, avoidance and hyperarousal) during the last month before assessment on a four-point Likert scale (0–3), with a symptom rated as present if the corresponding item has a score of ≥1. The 17 items are summarized by a total symptom severity score that ranges from 0 to 51, such that higher scores indicate greater symptom severity (mild severity, score ≤10; severe, score ≥36). The fourth part concerns impairments in different areas of life (e.g. job). The criteria for diagnosis of full PTSD were met if parents reported at least one intrusion symptom, three avoidance symptoms and two hyperarousal symptoms, as well as a duration of these symptoms of at least 1 month and impaired function in at least one life area [18]. For the diagnosis of partial PTSD, parents had to report fewer symptoms for each symptom cluster (e.g. at least one avoidance symptom compared to at least three avoidance symptoms which were required for the diagnosis of full PTSD), while the criteria for the duration of the symptoms and impaired function were the same [36]. In this study, HUS-related PTSD was analyzed in those parents who indicated that their child's HUS was the most stressful traumatic event. In the sample of parents assessed in this study, the internal consistency of the HUS-related PTSD symptom severity score was good (for the mothers: Cronbach's $\alpha = 0.83$; for the fathers Cronbach's $\alpha = 0.84$).

Socioeconomic status

Socioeconomic status was assessed with a demographic questionnaire and estimated according to maternal education and paternal occupation on a scale ranging from 2 to 12, with 2 being the lowest and 12 the highest SES score. Three social classes were assigned: lower (SES scores 2–5), middle (SES scores 6–9) and upper (SES scores 10–12). This measure has been proven to be a valid indicator of SES in previous studies involving the Swiss population [37].

Statistical analysis

Data were analyzed using the SPSS statistical package for Windows version 22.0 (IBM Corp., New York, NY). All tests were two-sided, and a p value of <0.05 was considered to be significant. Chi-square tests and Mann–Whitney U tests were used as appropriate to compare child sex, child age at HUS diagnosis, need for dialysis during acute HUS, length of hospital stay, development of ESRD and SES between participants and non-participants. Cronbach's alpha (α) was calculated to test the internal reliability of scale scores. HRQoL and mental health differences between the study sample and normative data were examined using one-sample t tests. Because most of the HRQoL and mental health scales as well as the PTSD total symptom severity scale showed non-normal distributions according to the Kolmogorov–Smirnov test, we used Wilcoxon signed-rank tests for testing the equality of means between mothers and fathers. For all comparisons, effect sizes were computed with Cohen's d (small effect 0.20; medium effect 0.50; large effect >0.80; study group vs. normative data; mothers vs. fathers) [38]. Multiple linear regression models were used to predict parental HRQoL using the MCS as the dependent variable. Due to non-normal distribution of the residuals, the MCS of the mothers and fathers was reflected square-root transformed for the regression analysis [39], which resulted in normally distributed residuals according to regression plots (histograms of the standardized residuals and probability plots). For both parents, two blocks of predictors were entered into the regression analyses. Block 1 (child medical characteristics) comprised type of HUS (atypical vs. typical HUS), time since acute HUS (years), dialysis during acute episode (yes vs. no), duration of hospital stay during acute episode (days) and chronic kidney disease (yes vs. no). Block 2 (child sociodemographic factors) comprised female sex, age at assessment of parental data (years) and SES (upper vs. middle/lower SES class). Selection of predictors was based on their assumed clinical relevance for parental HRQoL. The assumption of multi-colinearity was tested by checking the correlation matrix for high correlation coefficients (>0.80) and the variance inflation factor, which was not violated [40].

Results

Sociodemographic characteristics of children and parents

At assessment of parental data, the 63 HUS-affected children (56 % females) had an average age of 9.3 (SD 4.6, median 9.6, range 1.1–17.9) years. The mean age of the mothers and fathers was 41.1 (SD 6.3, median 41.5, IQR 36–46) years and 44.3 (SD 6.7, media 45.0, IQR 40–48) years, respectively. The SES of the participating families was categorized as lower class (4 families, 6 %), middle class (43, 68 %) and upper class (16, 26 %).

Diagnostic and medical characteristics of the children

Descriptive diagnostic and medical data of the HUS-affected children are shown in Table 1. The mean time for the pediatric patient group since an acute episode of HUS was 6.4 (SD 4.4, media 6.1, range 0.1–16.5) years. The predominant type of HUS was STEC-HUS, with verification of Shiga toxin in 35 cases. Of the 53 children with STEC-HUS, 36 (68 %) required dialysis during the acute phase of HUS. P-HUS was diagnosed in seven children (11 %), of whom one had meningitis, five had pneumonia and one had pneumonia and peritonitis; five of these seven children required dialysis during the acute phase of HUS. aHUS was diagnosed in three of the 63 children (5 %), with one or more complement-related mutations found in two of these three children. All three aHUS patients required acute dialysis, with two of them additionally requiring chronic dialysis.

Twenty-four of the patients (38 %) had CKD (18 with a STEC-HUS episode, 3 with a P-HUS episode, and 3 with aHUS). Most of these children were under medical supervision or required specific renal treatment. Of these 24 children, five had ESRD, and four of these five children had already undergone renal replacement (STEC-HUS, $n=1$; P-HUS, $n=1$; aHUS, $n=2$); one child with STEC-HUS was still on the waiting list for preemptive renal replacement at the time of assessment.

HUS-related long-term extrarenal sequelae were observed in three of the 24 patients with CKD. There was one case each of insulin-dependent diabetes mellitus due to STEC-HUS-related pancreatitis; severe neurodevelopmental impairment secondary to neurological involvement during acute STEC-HUS; impaired visual acuity due to retinal bleeding during acute STEC-HUS. In addition, three CKD patients were affected by another chronic disease unrelated to HUS (e.g. thoracic lymphangioma, hearing disorder and complications due to prematurity).

Health-related quality of life

Mean values of the HRQoL scales of the mothers and fathers and statistics for comparison of the study sample with normative data are presented in Table 2. For the mothers, all HRQoL subscales and the two component summary scales (MCS and PCS) were above the population norm, with the mothers of HUS-affected children showing better HRQoL than the population sample. Among fathers, better HRQoL scores than for male references were reported for physical function, role limitations due to physical problems, bodily pain, general health, emotional problems and PCS. For the other subscales and the MCS, paternal HRQoL was comparable to the normative data. Differences between the study sample and normative data were found with medium effect sizes for both mothers and fathers with respect to bodily pain, general health and PCS. Significant differences between mothers and fathers with small effect sizes were only found for role limitations caused by physical problems, which were less common in the fathers.

Table 1 Diagnostic and medical characteristics of children with hemolytic uremic syndrome($n = 63$)

Diagnostic and medical characteristics	Values
Classification of HUS type	
STEC-HUS	53 (84 %)
P-HUS	7 (11 %)
aHUS	3 (5 %)
Data on acute episode of HUS	
Child age at diagnosis (years)	3.0 ± 3.1
Time since acute HUS (years)	6.4 ± 4.4
Dialysis	44 (70 %)
Peritoneal dialysis	33 (75 %)
Hemofiltration/Hemodialysis	7 (16 %)
Combination of peritoneal dialysis and hemofiltration/hemodialysis	4 (9 %)
Duration of dialysis (days)	10.6 ± 14.5 [0–79]
On dialysis at time of discharge	2 (3 %)
Stay in intensive care unit	57 (91 %)
Duration of stay in intensive care unit (days)	7.81 ± 6.8 [0–31]
Duration of hospital stay (days)	23.7 ± 17.4 [5–97]
Clinical course of HUS at time of assessment	
Chronic kidney disease	24 (38 %)
Stage 1	9 (38 %)
Stage 2	10 (42 %)
Stage 3	0 (0 %)
Stage 4	0 (0 %)
Stage 5	5 (20 %)
Treatment modalities of the chronic kidney disease patients	
Conservative medical treatment	8 (33 %)
Plasmapheresis (and conservative treatment)	1 (4 %)
Renal transplantation	4 (17 %)
Medical supervision	11 (46 %)
HUS-related long-term extrarenal sequelae,	3 (5 %)

Data are presented as the mean ± standard deviation (SD), with/without the range in square brackets, or as a number with the percentage in parenthesis

HUS, Hemolytic uremic syndrome; STEC-HUS, Shigatoxin-associated HUS; P-HUS, *Streptococcus pneumoniae*-associated HUS; aHUS, atypical HUS

Prediction of parental HRQoL by HUS-related and sociodemographic characteristics

The statistics for the block-wise multiple regression analyses predicting parental HRQoL (MCS) are summarized in Table 3. Among mothers and fathers, the overall statistical model was not significant, accounting for 16 % of the total variance in mothers and 14 % in fathers. Maternal and paternal HRQoL did not differ with respect to their child's HUS type (aHUS, P-HUS, STEC-HUS). However, a shorter time since the child suffered an acute episode of HUS was a significant predictor of lower maternal HRQoL (MCS; the negative relationship between MCS and the time since diagnosis is valid for the transformed MCS, while for the original variable the relationship is positive). This effect was not present for the fathers.

Mental health

Mean values of maternal and paternal mental health scales and statistics for comparisons of the study sample with normative data are presented in Table 4. Statistical analyses revealed fewer psychopathological symptoms with small effect sizes in the parents than in normative data with respect to the Global Severity Index. Furthermore, both parents reported significantly fewer obsessive–compulsive symptoms, interpersonal sensitivity and less anxiety than gender-matched norms. In contrast to the fathers, mothers also reported significantly fewer symptoms of depression and phobic anxiety than female references. Differences between mothers and fathers with small effect sizes were found for depression and phobic anxiety, with fathers reporting more problems.

Prediction of parental mental health

The overall statistical model was not significant for mothers and fathers, accounting for 9 % of the total variance in mothers and 11 % in fathers. None of the selected predictors for lower parental mental health (Global Severity Index for mothers and fathers) was significant.

Table 2 Health-related quality of life of mothers and fathers with HUS-affected children, compared with population norm and between mothers and fathers

SF-36 scales	Mothers ($n = 61$–63)	Comparison of mothers' HRQoL with female references (normative data)[a]		Fathers ($n = 58$)	Comparison of fathers' HRQoL with male references (normative data)[a]		Comparison of mothers' and fathers' HRQoL[b] ($n = 56$ –58)	
		p	d[c, d]		p	d[c, d]	p	d[c, e]
Physical functions	94.4 ± 16.5	0.02	0.30	96.2 ± 8.0	<0.001	0.44	0.88	−0.14
Role physical	91.1 ± 20.9	0.01	0.27	97.4 ± 10.1	<0.001	0.46	0.04	−0.38
Bodily pain	83.8 ± 22.0	<0.001	0.77	90.0 ± 16.6	<0.001	0.88	0.09	−0.32
General health	80.8 ± 16.1	<0.001	0.69	77.6 ± 16.1	<0.001	0.59	0.30	0.20
Vitality	65.7 ± 17.1	<0.001	0.48	67.1 ± 17.2	0.20	0.17	0.73	−0.08
Social functions	89.3 ± 18.5	0.05	0.24	90.7 ± 15.6	0.46	0.09	0.49	−0.08
Role emotional	94.5 ± 20.4	<0.01	0.30	96.0 ± 15.4	0.05	0.20	0.52	−0.08
Mental health	77.4 ± 14.9	<0.001	0.47	76.7 ± 15.9	0.48	0.10	0.82	0.04
PCS	54.3 ± 7.6	<0.001	0.70	55.4 ± 4.7	<0.001	0.81	0.70	−0.18
MCS	52.1 ± 8.8	<0.01	0.34	51.6 ± 8.2	0.85	−0.02	0.78	0.06

Data on health-related quality of life (HRQoL) are presented as the mean ± SD. The higher the value, the better the HRQoL

SF-36, Medical Outcomes Study Short Form-36 item questionnaire; PCS physical component summary score of SF-36; MCS, mental component summary score of SF-36

[a] Values are presented as the mean ± SD. One-sample t tests were performed

[b] Wilcoxon signed-rank tests were performed

[c] d = Effect size according to Cohen [38], with a Cronbach's α value of 0.20 indicating a small effect; an α value of 0.50 indicating a medium-sized effect; an α value of >80 indicating a large effect)

[d] Differences: Study sample vs. normative data (positive d indicates better HRQoL for the study sample compared to the population norm)

[e] Differences: Mothers' vs. fathers' data (positive d indicates better HRQoL for the mothers than for the fathers)

Table 3 Prediction of parental health-related quality of life[a] by multiple regression analysis of the medical and sociodemographic characteristics of the HUS-affected child ($n = 61$)

Medical and sociodemographic characteristics	Mothers ($n = 61$)		Fathers ($n = 58$)	
	ΔR^2	β	ΔR^2	β
Block 1: Medical characteristics	0.074		0.089	
Atypical HUS		−0.03		0.05
Time since acute HUS		−0.44*		0.20
Dialysis during acute episode		−0.18		−0.08
Duration of hospital stay during acute episode		0.02		−0.28
Chronic kidney disease		0.12		0.19
Block 2: Sociodemographic characteristics	0.087			
Female sex		0.15		0.21
Age at assessment		0.29		0.04
Upper SES class		0.13		0.07
Total R^2	0.161		0.137	
Total R^2 adjusted	0.032		−0.004	

*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

ΔR^2 , Change in multiple regression coefficient; β standardized regression coefficient; SES socioeconomic status

[a] Health-related quality of life (HRQoL) assessment was based on the mental component summary (MCS) score of the SF-36, where the MCS score is one of two standardized component summary scores of the SF-36. For both mothers and fathers MCS was transformed. For interpretation, the direction of ß has to be reversed; for actual use of the regression equation, the transformed MCS has to be backtransformed

Posttraumatic stress disorder

Overall, 48 of the 63 mothers (76 %) and 41 of the 58 fathers (71 %) reported some traumatic experience, and the criteria for full PTSD (due to any traumatic events) were met by three mothers (5 %) and two fathers (3 %). The HUS of their respective child was stated as a single and/or the most troubling traumatic experience by 24 of the 48 mothers (50 %) and by 20 of the 41 fathers (49 %). Descriptive information with respect to the number of HUS-related PTSD symptoms and the HUS-related symptom severity scales is given in Table 5. None of the mothers (0 %) met the criteria for full HUS-related PTSD, while four mothers (6 %) met the criteria for partial HUS-related PTSD. Among the fathers, one (2 %) and two fathers (4 %) met the criteria for partial HUS-related PTSD and full HUS-related PTSD, respectively.

Discussion

This cross-sectional study provides a comprehensive insight into self-reported HRQoL and mental health in relation to PTSD symptoms in parents of HUS-affected children. Our results show that neither HRQoL nor mental health was impaired in the mothers or the fathers of our study sample compared to normative data, while full HUS-related PTSD was diagnosed in two fathers but no mothers.

In contrast to our hypothesis, parental HRQoL was comparable with or even greater than the population norm. This result is consistent wit those from a number of previously published studies on HRQoL in parents of children with other life-threatening or chronic diseases (e.g. meningococcal septic shock [23], Kawasaki syndrome [24] or juvenile iodiopathic arthritis [41]). However, it differs from the finding of one study of parents of children with ESRD where the parents had a lower HRQoL than healthy references (e.g., with respect to depressive symptoms and vitality) [13]. The findings of a study on parents of children with advanced CKD also differ from our results [14]. One possible explanation for our results is the small number of children with ESRD or advanced CKD. Furthermore, methodological differences between the available studies with parents of pediatric HUS patients (e.g. the use of different HRQoL questionnaires) make it difficult to compare the results. However, the greatest differences in HRQoL (medium effect sizes) between the study sample and the population norm were found for maternal and paternal bodily pain, general health and the PCS. We can therefore speculate that parents of HUS-affected children rate their own physical health as better than the norms because they rate their health in comparison to that of their sick child. Our study also indicated that a shorter time since an acute episode of HUS was a significant predictor of lower maternal HRQoL, which is consistent with previous studies of parents with children having other chronic diseases (e.g., heart disease) and also with a phase model of adjustment of medical stress as

Table 4 Mental health of mothers and fathers with HUS-affected children, compared with population norm and between mothers and fathers

Brief Symptom Inventory scales	Mothers ($n = 63$)	Comparison of mothers' mental health scores with normative data[a]		Fathers ($n = 58$)	Comparison of fathers' mental health scores with normative data[b]		Comparison of mothers' and fathers' mental health scores[b]	
		p	d [c, d]		p	d [c, d]	p	d [c, e]
Somatization	47.8 ± 8.9	0.05	−0.23	48.0 ± 9.3	0.10	−0.21	0.79	−0.02
Obsessive-compulsive	46.6 ± 10.4	0.01	−0.33	46.9 ± 10.4	0.03	−0.30	0.37	−0.03
Interpersonal sensitivity	46.7 ± 10.8	0.02	−0.32	47.6 ± 8.5	0.04	−0.26	0.43	−0.09
Depression	46.6 ± 9.7	<0.01	−0.35	49.3 ± 8.9	0.53	−0.08	0.01	−0.29
Anxiety	47.4 ± 10.0	0.05	−0.26	47.3 ± 9.8	0.04	−0.27	0.68	0.02
Hostility	49.3 ± 10.7	0.58	−0.07	49.5 ± 9.5	0.70	−0.05	0.87	−0.03
Phobic anxiety	47.4 ± 7.4	<0.01	−0.30	48.4 ± 7.4	0.11	−0.18	<0.001	−0.14
Paranoid ideation	48.1 ± 9.4	0.12	−0.19	50.5 ± 9.9	0.71	0.05	0.06	−0.24
Psychoticism	50.8 ± 10.2	0.55	0.08	48.2 ± 7.8	0.08	−0.20	0.50	0.29
Global Severity Index	45.0 ± 13.2	<0.01	−0.43	45.0 ± 13.5	<0.01	−0.42	0.96	−0.01

Data on the mental health of the parents are presented as the mean ± SD. The higher the value, the lower the mental health score

[a] Wilcoxon signed-rank tests were performed

[b] One-sample t tests were performed

[c] d = Effect size according to Cohen [38], with a Cronbach's α value of 0.20 indicating a small effect; an α value of 0.50 indicating a medium-sized effect; an α value of >80 indicating a large effect)

[d] Differences: study sample vs. normative data (negative d indicates better mental health for the study sample compared to the population norm)

[e] Differences: Mothers' vs. fathers' data (negative d indicates better mental health for the mothers than for the fathers)

suggested by Kazak et al. [42, 43]. This model describes three consecutive phases of traumatic stress responses to medical events, with increasing traumatic stress during phase I (also called Peri-Trauma which includes the initial traumatic event and surrounding events as, for example, invasive medical procedures).

In our study, maternal and paternal mental health as measured by the BSI was not impaired compared to normative data. This is in contrast to previous reports on the psychological distress of parents with children affected by HUS associated with verocytotoxin-producing *Escherichia coli* [17] and by various chronic diseases [12, 44]. However, in our study only 24 of the 63 children (38 %) had CKD, which might partly explain our result. Furthermore, we found no significant association between HUS-related or sociodemographic characteristics and parental mental health in our study. Our finding might be also explained by a so-called response shift [45], such that parents might have adapted to the new situation over time and are therefore satisfied with less compared to the normal population. It might also be that parents of the HUS-affected children received good medical care and adequate social support, which possibly might have mitigated parental distress. Another possibility is that parents might experience personal growth in relation to their child's HUS and the difficulties associated with it [46, 47]. However, we found that some parents—even 6.4 years after the acute HUS episode—were affected by full or partial PTSD due to their child's HUS. In fact, it is possible that parents simply do not report mental health problems as measured by the BSI and the presence of PTSD symptoms. The BSI does not ask about such symptoms. Our finding should be of great interest to nephrologists and other healthcare providers caring for HUS-affected children and their families during the clinical follow-up. Assessing and treating any posttraumatic stress symptoms in these parents may prevent or at least mitigate any negative consequences of PTSD and may also help maintain their competency as caregivers [48].

This study is the first to report HRQoL and mental health data of parents with HUS-affected children. Its strengths include the use of standardized multidimensional questionnaires and the comparison of results with normative data. Furthermore, a high proportion of fathers (92 % of participating families) participated in our study, allowing comparisons between maternal and paternal HRQoL and mental health scales. Nevertheless, our study has several limitations. First, our study sample comprised different HUS types characterized by some heterogeneity with respect to HUS etiology, pathophysiology and the clinical course of the disease. Thus, while we were

Table 5 Prevalence of post-traumatic stress symptoms and post-traumatic stress disorder (PTSD) according to DSM-IV in 63 mothers and 58 fathers experiencing their child's hemolytic uremic syndrome (HUS) as a traumatic event

Posttraumatic Diagnostic Scale items	Mothers	Fathers	Comparison between mothers and fathers[a]	
			p	*d*[b]
Parents stating their child's HUS as a traumatic event	24 (38 %)	20 (35 %)		
Intrusion				
DSM-IV criterion met	20 (32 %)	10 (17 %)		
Number of symptoms	2.1 ± 1.5 [0–5]	1.2 ± 1.4 [0–4]	0.02	0.65
Avoidance				
DSM-IV criterion met	1 (2 %)	2 (4)		
Number of symptoms	0.9 ± 1.4 [0–6]	0.6 ± 1.3 [0–5]	0.02	0.24
Hyperarousal				
DSM-IV criterion met	7 (11 %)	6 (10 %)		
Number of symptoms	1.1 ± 1.5 [0–5]	0.9 ± 1.2 [0–3]	0.11	0.17
All 3 symptom clusters				
DSM-IV criteria for full PTSD met	0 (0 %)	2 (4)		
Criteria for partial PTSD met	4 (6 %)	1 (2)		
Total symptom severity	6.1 ± 6.7 [1–30]	3.1 ± 4.0 [0–15]	<0.01	0.54

Data are presented as the mean ± SD with the range given in square brackets, or as a number with the percentage in parenthesis

PTSD, Post-traumatic stress disorder; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (version IV)

[a] Wilcoxon signed-rank tests for continuous variables were performed

[b] *d* = Effect size according to Cohen [38], with a Cronbach's α value of 0.20 indicating a small effect; an α value of 0.50 indicating a medium-sized effect; an α value of >80 indicating a large effect). Mothers' vs. fathers' data, with a positive *d* indicating a higher number of PTSD symptoms for the mothers than for the fathers

able to present a good overview of the broad clinical HUS spectrum, our study included a rather small number of children with aHUS, making it difficult to compare parental HRQoL and mental health issues with respect to the type of pediatric HUS. However, our multivariate regression analysis revealed that the type of HUS was not a significant predictor for parental HRQoL. Secondly, our data were compared with gender-matched normative data that might be more or less than sufficient. We compared our data with normative data within the same inter-quartile age ranges, but not with exactly age-matched controls. Since it is well known that age has an influence on HRQoL and mental health changes [30], this methodological issue might have influenced our results. However, our study provides a good insight into the issues which are most strongly affected and which should be carefully considered in clinical practice.

In conclusion, the results of our study indicate that HRQoL and mental health in parents of children with a history of HUS were comparable with or better than normative data. However, a few parents of our study sample suffered from full or partial PTSD due to their child's HUS. Although the prevalence of HUS-related PTSD was small, special attention should be given to parental PTSD symptoms in the clinical follow-up of HUS-affected children, since even partial PTSD might lead to functional impairment [36] and some parents would probably benefit from psychological support.

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Compliance with ethical standards The study was approved by the Ethics Committee of the Canton of Zurich and registered at ClinicalTrials.gov (NCT 01666548). Written informed consent was received from parents.

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Conflict of interest The authors declare no conflicts of interest.

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Original Article

ORIGINAL ARTICLE

Membranoproliferative glomerulonephritis and C3 glomerulopathy in children: change in treatment modality? A report of a case series

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Abstract

Background: Membranoproliferative glomerulonephritis (MPGN) with immune complexes and C3 glomerulopathy (C3G) in children are rare and have a variable outcome, with some patients progressing to end-stage renal disease (ESRD). Mutations in genes encoding regulatory proteins of the alternative complement pathway and of complement C3 (C3) have been identified as concausative factors.

Methods: Three children with MPGN type I, four with C3G, i.e. three with C3 glomerulonephritis (C3GN) and one with dense deposit disease (DDD), were followed. Clinical, autoimmune data, histological characteristics, estimated glomerular filtration rate (eGFR), proteinuria, serum C3, genetic and biochemical analysis were assessed.

Results: The median age at onset was 7.3 years and the median eGFR was 72 mL/min/1.73 m^2. Six children had marked proteinuria. All were treated with renin–angiotensin–aldosterone system (RAAS) blockers. Three were given one or more immunosuppressive drugs and two eculizumab. At the last median follow-up of 9 years after diagnosis, three children had normal eGFR and no or mild proteinuria on RAAS blockers only. Among four patients without remission of proteinuria, genetic analysis revealed mutations in complement regulator proteins of the alternative pathway. None of the three patients with immunosuppressive treatment achieved partial or complete remission of proteinuria and two progressed to ESRD and renal transplantation. Two patients treated with eculizumab revealed relevant decreases in proteinuria.

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Conclusions: In children with MPGN type I and C3G, the outcomes of renal function and response to treatment modality show great variability independent from histological diagnosis at disease onset. In case of severe clinical presentation at disease onset, early genetic and biochemical analysis of the alternative pathway dysregulation is recommended. Treatment with eculizumab appears to be an option to slow disease progression in single cases.

Key words: C3 glomerulopathy, complement dysregulation, eculizumab, MPGN, paediatrics

Introduction

Membranoproliferative glomerulonephritis (MPGN) with immune complexes is a rare chronic glomerulonephritis in childhood characterized by proteinuria (up to the nephrotic range), haematuria, hypertension and often impaired renal function at disease onset [1]. In up to 50% of affected children, MPGN leads to renal failure within 10 years [2]. Impaired renal function after 1 year of onset is considered a risk factor for poor renal outcome and end-stage renal disease (ESRD) [1]. The recurrence rate after renal transplantation (RTPL) is high (up to 45%) [1–4]. MPGN may occur as a primary genetic disorder or secondary to chronic diseases, including infections (e.g. hepatitis B or C), systemic lupus erythematosus, liver disease and malignancies.

In the past, MPGN was diagnosed and classified by renal histological features and grouped into three pathological subtypes with different aetiologies and pathogenesis, types I, II [(dense deposit disease (DDD)] and III [5]. Activation of the alternative complement pathway has repeatedly been observed in conjunction with low serum levels of complement C3 (C3) [6–8]. A link between dysregulation of the alternative complement pathway and the pathogenesis of MPGN was assumed [7] and has recently been confirmed by findings of mutations in the genes of complement factor H (CFH) and CF-related proteins (CFHR) in DDD [9–13].

Therefore the histological classification has been reconsidered recently on the basis of pathogenesis and with division into those cases in which the glomerular immune deposits stain for immunoglobulins and complement and those cases that are characterized by C3 deposition alone [5, 14–16]. The term 'C3 glomerulopathy' (C3G) encompassed complement-mediated renal disease, and therefore incorporates disease entities where the presence of a disease-associated complement mutation is causally associated with the underlying renal pathology. Examples include DDD and C3 glomerulonephritis (C3GN) [5]. The term C3GN was coined to describe glomerular lesions in which there is glomerular accumulation of C3 with little or no immunoglobulin in the absence of the characteristic highly electron-dense transformation seen in DDD [16]. The incidence of C3G is estimated to be 1–2 per 10^6 children [17, 18], with disease recurrence after RTPL reported at between 30 and 77% and a graft failure due to recurrence in 17–50% of the recipients [19, 20]. MPGN associated with the presence of immunoglobulins and complement has been termed immune complex–mediated MPGN by Sethi and Fervenza [21]. Immune complex-mediated MPGN is commonly associated with autoimmune disease and chronic infection and can be associated with mixed cryoglobulinaemia or monoclonal gammopathy [5, 21]. These associations were excluded in our study. In our series, histological MPGN I is a case of so-called idiopathic MPGN. MPGN I and C3G are regarded as heterogeneous diseases, with several studies reporting complement mutations in complement genes [15, 22]. So far there are no evidence-based guidelines for treatment of MPGN I [1, 2] and C3G. The mainstay of treatment in MPGN I and C3G is based on single-centre studies and expert opinions. Clinical trials in adults and children with different treatment modality propositions [e.g. immunosuppressant agents, antiplatelet drugs and plasmapheresis (PEX)] are described [23–29]. Treatment with renin–angiotensin–aldosterone system (RAAS) blockers are described to induce a decrease in proteinuria and delays progression to ESRD in many glomerular diseases in adults [30, 31] and in some glomerulopathies in children [32, 33]; however, there is no evidence for beneficial effect in children with MPGN I [34] and spontaneous recovery in C3GN can also not be excluded [22].

Eculizumab is a monoclonal antibody binding to C5 and thereby inhibiting the complement system and preventing activation of the alternative pathway. Recently, promising results were shown in selected patients with MPGN and C3GN characterized by the presence of alterations in regulatory proteins of the alternative complement pathway [22, 35–37].

We evaluated the outcome of seven children with the diagnosis of MPGN I, C3GN and DDD. All were screened for the presence of genetic mutations of the alternative complement pathway. We analysed the benefit of different administered treatments.

Materials and methods

All patients with histologically diagnosed MPGN I, DDD and C3GN who were seen at the University Children's Hospital Zurich between 2003 and 2015 were included in a retrospective data analysis. Clinical assessment included diagnosis, sex, medical history, clinical examination, blood pressure (BP) and analysis to exclude potentially secondary forms of MPGN.

Biochemical and genetic analysis

Plasma creatinine, total serum protein and albumin and urinary protein:creatinine ratio (UPCR) were analysed at disease onset and at the last follow-up. Blood samples for complement regulator protein analysis were centrifuged immediately after collection and frozen at −80°C prior to analysis. C3 serum levels were evaluated by kinetic nephelometry (Immage 800, Beckman Coulter, Brea, CA, USA) with a normal value >0.7 g/L (reference value of the immunology laboratory of Zurich University Children's Hospital). The soluble complement components C5b-9 (membrane attack complex), C3d and C3 nephritic factor (C3NeF) were measured from plasma samples at the Institute for Immunology of Heidelberg University (Heidelberg, Germany). The following biochemical analyses were performed at follow-up from plasma and serum, as well as ethylenediaminetetraacetic acid blood samples for genetic analysis (samples at the Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany): CFH antibody, C3-convertase antibody, CFI, CFB, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, MCP (CD46) and by next-generation sequencing (NGS) at the Center for Human Genetics at Bioscientia in Ingelheim (Germany), as described in detail below.

Targeted NGS using a customized multigene panel for atypical hemolytic uremic syndrome (aHUS) and related disorders was performed in all patients [38]. In brief, we utilized a customized sequence capture library that targets exons and additionally 35 bp of flanking intronic sequence (20–23). Genomic DNA was fragmented and the coding exons of the analysed genes, as well as the corresponding exon–intron boundaries, were enriched using the Roche/NimbleGen sequence capture approach (NimbleGen, Madison, WI, USA), amplified and sequenced simultaneously by Illumina NGS sequencing-by-synthesis technology using a HiSeq 1500 system (Illumina, San Diego, CA, USA). Target regions were usually sequenced with an average coverage of ∼400–500-fold. With this method, 20-fold coverage is obtained for >99.5% of the regions of interest. NGS data analysis was performed using bioinformatic analysis tools and JSI Medical Systems software (version 4.1.2; JSI Medical Systems, Kippenheim, Germany). Identified variants and indels were filtered against external and internal databases and depending on allelic frequency. The focus was on rare variants with a minor allele frequency of ≤1%. Nonsense, frameshift and canonical splice site variants were considered a priori as likely to be pathogenic. Pathogenicity of identified non-synonymous variants was assessed using bioinformatic prediction programs, such as Mutation Taster, Polyphen-2, MutationAssessor and FATHMM. Only those variants predicted by the majority of algorithms used to be probably damaging were considered likely to be pathogenic. In silico analysis of splice site effects was performed using bioinformatic programs such as Fruitfly, NetGene2, Human Splicing Finder, Mutation Taster and ESEFinder. Mapping and coverage statistics were generated from the mapping output files using GATK. The resulting sequence data were compared with the reference sequence of the RefSeq database. High coverage enabled copy number variation analysis. Potential copy number alterations were initially identified by VarScan on mapped reads. In this way, coverage of every target region of the sample was internally normalized and compared with normalized control data of other samples of the same run by VarScan copy number mode and standard settings. Putative pathogenic differences between the wild-type sequence (human reference genome according to the University of California, Santa Cruz Genome Browser: hg19, GRCh37) and the patient's sequence were validated by conventional Sanger sequencing and, in the case of copy number variation, by multiplex ligation-dependent probe amplification.

Renal biopsy

All patients underwent native kidney biopsy at disease onset. Repeated renal biopsy was performed in two children, one of the native kidneys and one in the transplanted kidney. The biopsy diagnosis was based on the current classification and nomenclature for C3G and MPGN [5, 16] on findings by light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM).

Definitions

The following definitions were used: nephrotic-range proteinuria: UPCR >250 g/mol; nephrotic syndrome: serum albumin <25 g/L, nephrotic-range proteinuria and generalized oedema; remission: UPCR <20 g/mol; partial remission: UPCR >20–<80 g/mol; normal renal function: estimated glomerular filtration rate (eGFR) >90 mL/min/1.73 m^2, as calculated by the Schwartz formula using a local k-factor of 40 [39]; hypertension: casual systolic BP >95th percentile for sex and height [40].

Treatment

All patients were given RAAS blockers as first-line antiproteinuric therapy. Additional treatment consisted of prednisolone (PDN), in accordance with the dosage regimen for treatment of children with steroid-resistant nephrotic syndrome [41, 42]. Further immunosuppressive treatment [cyclosporine A (CSA) and mycofenolate mofetile (MMF)] [25, 42] was administered to patients who failed to attain partial or full remission of proteinuria. Eculizumab has been available in our hospital since 2013, but costs were not covered by all insurances, as this drug is still off-label for MPGN/C3G treatment. Eculizumab was given to two patients with nephrotic-range proteinuria despite immunosuppressive and RAAS blocker treatment. The dosage followed current recommendations for aHUS [43]. Patients treated with eculizumab were immunized with meningococcal vaccine (Menveo, GlaxoSmithKline Biologicals, Rixensart, Belgium) 4 weeks prior to administration. Informed consent was obtained from all parents of included patients for medical data collection, genetic analysis and treatment with eculizumab.

Results

Seven children (four boys and three girls) fulfilled the inclusion criteria (Table 1): three patients suffered from MPGN I, three from C3GN and one from DDD. The median age at onset was 7.3 years (range 2.5–12.5) with a median eGFR of 72 mL/min/1.73 m^2 (range 41–140) at diagnosis.

Table 1. Patient characteristics at disease onset

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)	7.3	2.5	6	12.5	5.9	10.5	8.8
Sex	Male	Male	Female	Female	Male	Female	Male
Hypertension	No	No	No	No	No	Yes	Yes
Haematuria	Micro	Micro	Macro	Micro	Macro	Micro	Micro
UPCR (g/mol) (reference <20)	634	126	530	855	580	1500	1000
Nephrotic syndrome (UPCR >250 g/mol)	No	No	No	Yes	No	Yes	Yes
Serum albumin (g/L) (reference 35–51)	27	32	26	11	30	12	12
eGFR (mL/min/1.73 m^2) (reference >90)	72	140	41	67	88	65	95

Table 2. Genetic, complement and histology testing

Test		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Genetic testing	CFHR1					CFHR1 c.880G>A (p.Glu294Lys) het; CFHR1/3 del het	CFHR1/3 del het	CFHR1/3 del het
	CFHR2				CFHR2 c.109G>A (p.E37K) het; c.584G>C(pG195A) het			
	CFHR5		CFHR5: c970 + 2T>G het					
	Factor H				CFH-het (=two functional polymorphisms)			
	MCP	MCP: c.989–78G>A hom, c.*897T>C hom (hom risk haplotype MCPggaac)	MCP: het risk haplotype MCP-H1	MCP: het risk haplotype MCPggaac	MCP: het risk haplotype MCPggaac	MCP: het risk haplotype MCP-H1	MCP: hom risk haplotype MCP-H1	MCP: het risk haplotype MCP-H1
C3 antibody positivity		No	No	No	No	No	Yes	Yes
C3NeF		Negative	Negative	Negative	Negative	Positive[a]	Negative	Negative[a]
C3 g/L (reference 0.7–1.76)[b]		0.4	<0.06	0.23	0.26	0.3	0.24	**0.6**
Renal histology biopsy		MPGN I	MPGN I	C3 GN	MPGN I	C3-GN	[c]C3-GN	DDD
LM		Mesangial and endocapillary proliferation. Double contours of GBM	Mesangial and endocapillary proliferation. Double contours of GBM	Mesangial and mild endocapillary proliferation, double contours of GBM, humps	Mesangial and mild endocapillary proliferation, double contours of GBM, crescents, 36% glomeruli hyalinized, 50% interstitial fibrosis and tubular atrophy.	Mesangial and mild endocapillary proliferation	Mesangial and endocapillary proliferation, double contours of GBM, 40% hyalinized glomeruli, segmental sclerosis, crescents, >95% interstitial fibrosis and tubular atrophy	Mesangial and mild endocapillary proliferation, 25% hyalinized glomeruli, crescents in 50% of glomeruli, thickended, glassy GBM
IF		Dominant GBM and mesangial positivity for IgG (2+) and C3 (2+)	Dominant GBM and mesangial positivity for C3 (3+), IgG (2+), IgM (3+)	GBM and mesangial positivity for C3 (3+), IgM (1+). IgG is negative	Dominant GBM and mesangial positivity for IgG (3+) and C3 (3+)	Dominant mesangial and GBM positivity for C3 (3+). IgG is negative	Mesangial and GBM positivity for C3 (3+), less IgG (1+)	GBM positivity for C3 (3+), IgM (3+), IgG (1+)
Electron microscopy		Subendothelial and mesangial electron-dense deposits	Subendothelial, mesangial and rare small subepithelial and intramembranous electron-dense deposits	Mesangial, subendothelial, intramembranous and subepithelial electron-dense deposits with humps	Mesangial, subendothelial, intramembranous and rare subepithelial electron-dense deposits	Granular, not very dense intramembranous and mesangial electron-dense deposits	Mesangial and intramembranous, not very dense granular electron-dense deposits. Rare small subepithelial deposits.	Highly osmiophilic segmental electron-dense deposits in lamina densa of GBM

Hom, homozygous; het, heterozygous; del, deletion; GBM, glomerular basement membrane. [a]Result performed only under treatment with eculizumab in Patient 5 and with PEX in Patient 7. [b]Values of serum complement C3 at disease onset. [c]Second biopsy 7 years after onset biopsy.

Analysis of the alternative complement pathway: CFs and genetic testing

All children showed persistently low serum C3 (Table 2). In addition, C3NeF was negative in all but one patient and C3b antibodies were positive in two children with C3GN.

At the last follow-up (Tables 3–5), C3d was elevated in five patients and sC5b-9 levels were increased in four children, both in MPGN I and C3GN.

Renal biopsy

Renal biopsy findings (MPGN I, C3GN and DDD) (Figures 1–4) are presented in Table 2. Repeated renal biopsy was performed in Patient 4 (native kidney, before embarking on MMF) and Patient 7 (renal graft for suspected acute graft rejection). The diagnoses were made based on IF findings. As the IF slides were no longer available, the figures contain pictures of immunohistochemistry performed for the purpose of the publication.

Treatment regimens and follow-up

The median follow-up was 9 years (range 2.2–11.5). Five children maintained renal function of their native kidneys, with a median eGFR of 107 mL/min/1.73 m^2 (range 40–180), including four patients with normal eGFR. Two children reached ESRD and underwent RTPL with initially good renal graft function. All children had BP $<$ 95th percentile, with six on RAAS blockers: in one child, RAAS blockers were stopped after RTPL. All children exhibited persistent haematuria. Three patients with nephrotic syndrome (Patients 4, 6 and 7) exhibited progression of proteinuria on PDN and were given additional immunosuppressive treatment.

Patients with RAAS blockers only

Patients 1–3 (MPGN I, $n = 2$; C3GN, $n = 1$) (Table 3). All had normal renal function at onset without signs of a nephrotic syndrome. Follow-up was uneventful, with partial or complete remission of proteinuria and maintained normal renal function. All had persistently low C3. Genetic testing revealed a risk haplotype for the membrane cofactor protein (MCP/CD46) gene in all three children. In addition, Patient 2 (MPGN I) had elevated sC5b-9 and a heterozygous mutation in the CFHR5 gene.

Patients with additional immunosuppressive treatments, including eculizumab

Patient 4 (MPGN I) (Tables 4). Because of progressive proteinuria on PDN, MMF was started [25]. Daily proteinuria initially decreased from 4 to 2 g. However, impaired renal function and nephrotic-range proteinuria persisted with elevated alternative pathway activity. A second renal biopsy confirmed the diagnosis of MPGN I. Therefore MMF was discontinued and eculizumab was started. Six months after starting eculizumab, a significant decrease in proteinuria and sC5b-9 was observed with stabilization of eGRF.

Patient 5 (C3GN) (Tables 4). Because of increasing proteinuria with normal renal function and persistently elevated alternative pathway activity despite RAAS blockade, the treatment

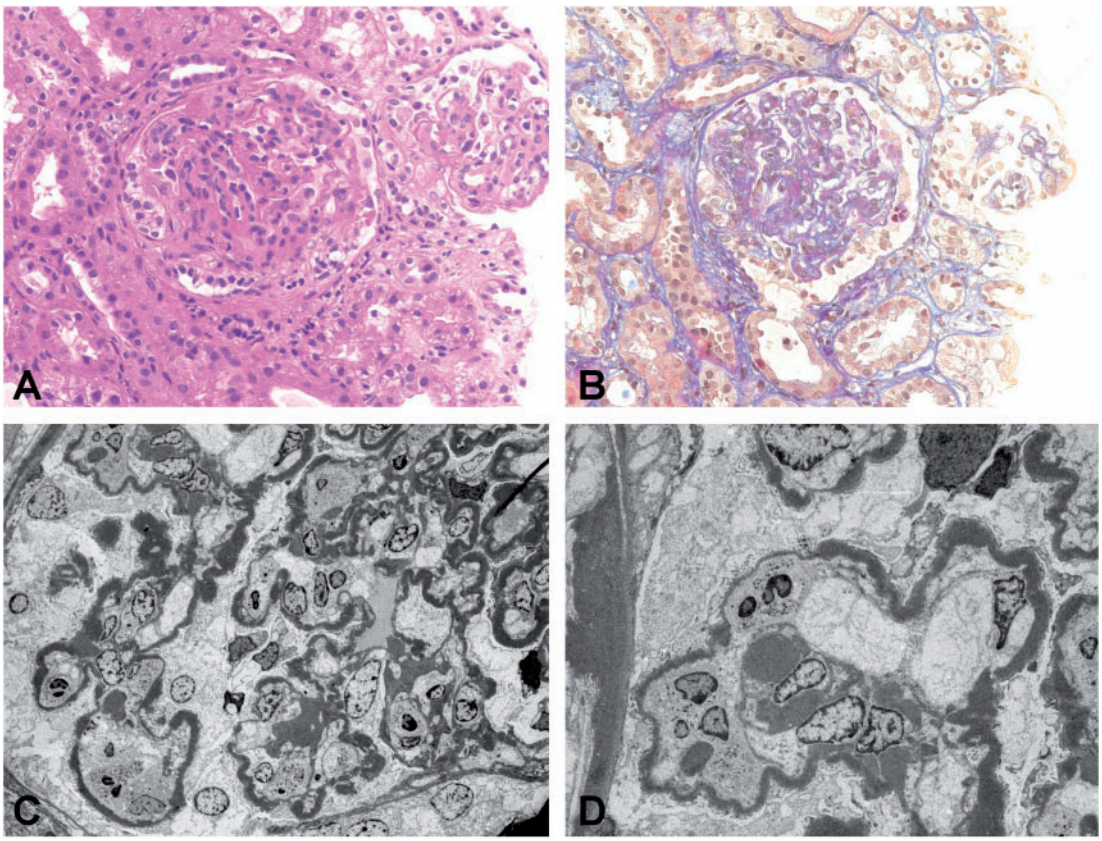

Fig. 1. DDD native kidney (Patient 7). (**A**) Glomeruli with mesangial and endocapillary proliferation and a fibrocellular crescent [hematoxylin and eosin (H&E) stain, original magnification ×200]. (**B**) Intramembranous and mesangial deposits (acid fuchsin orange G stain, original magnification ×200). (**C** and **D**) EM with highly osmiophilic electron-dense deposits in the lamina densa of the glomerular basement membrane and mesangium (original magnification ×1100 and ×1950).

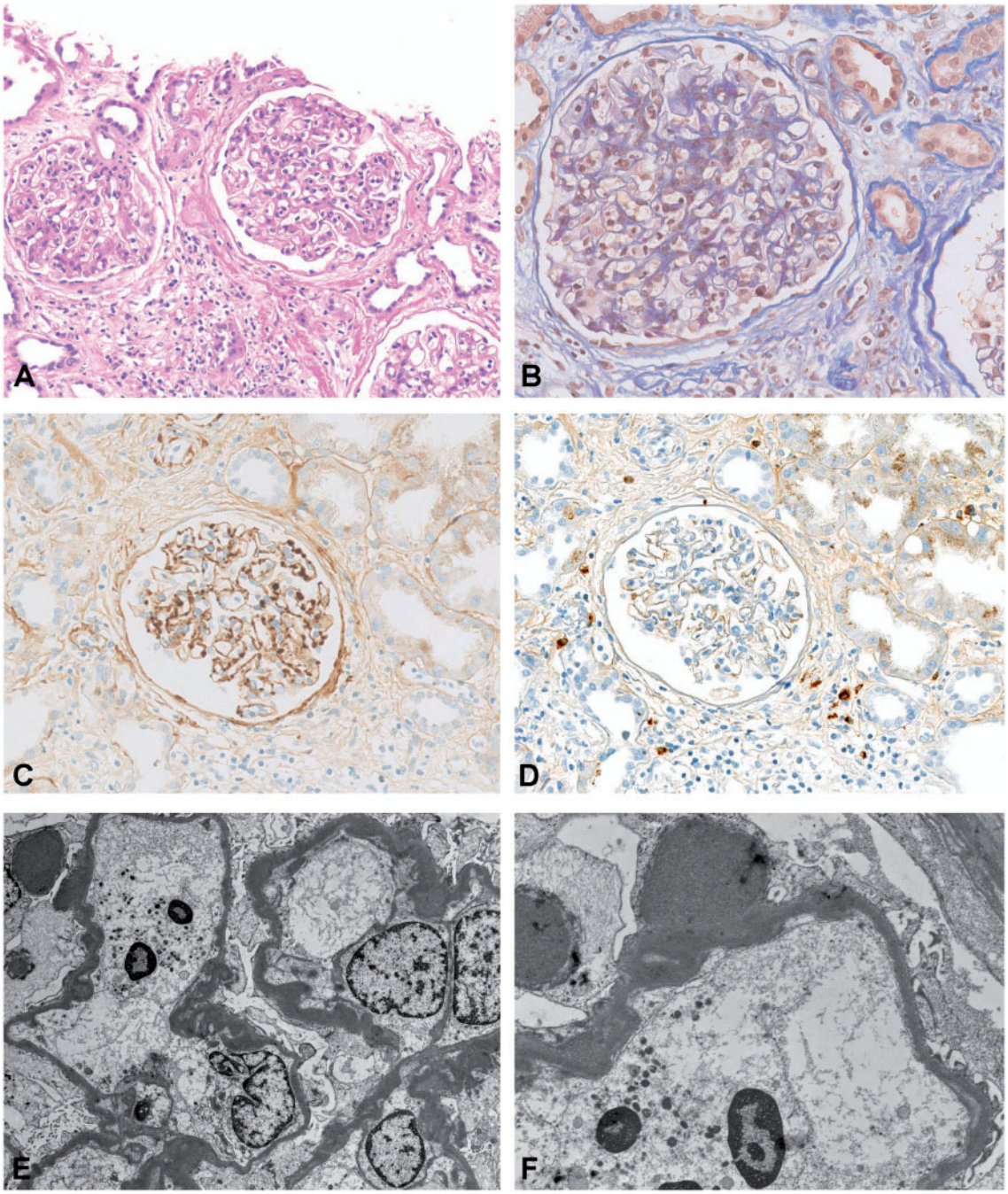

Fig. 2. DDD recurrence in transplant biopsy (Patient 7). (**A**) Thickened, glassy GBM and endocapillary hypercellularity with mononuclear cells (H&E stain, original magnification ×200). (**B**) Intramembranous deposits and endocapillary proliferation (AFOG stain, original magnification ×324). (**C**) Immunohistochemistry positive for C3 (original magnification ×200). (**D**) Immunohistochemistry negative for immunoglobulin G (original magnification ×200). (**E** and **F**) EM with highly osmiophilic electron-dense deposits in the lamina densa and subepithelial humps (original magnification ×1000 and ×2500).

with eculizumab was initiated [43]. After 11 months, proteinuria and sC5b-9 level decreased, whereas C3NeF remained positive. Renal function remained stable at a normal level.

Patient 6 (C3GN) (Table 5). Nephrotic-range proteinuria persisted despite additional treatment with CSA for 6 years. The child reached ESRD and underwent peritoneal dialysis 6.5 years after disease onset. Deceased-donor RTPL was performed; immunosuppression included tacrolimus, MMF, PDN and induction therapy with basiliximab. RAAS blocker was stopped. Seven months after transplantation, sC5b-9 was still slightly elevated. Renal graft function remained stable without proteinuria.

Patient 7 (DDD) (Table 5). Additional treatment with CSA was started due to persistent nephrotic-range proteinuria. The child reached ESRD 4.2 years after disease onset. Living paternal-donor RTPL was performed; immunosuppression included CSA, MMF and PDN. The child experienced acute/active antibody-mediated rejection (aABMR) and recurrence of DDD in the graft 3.3 years

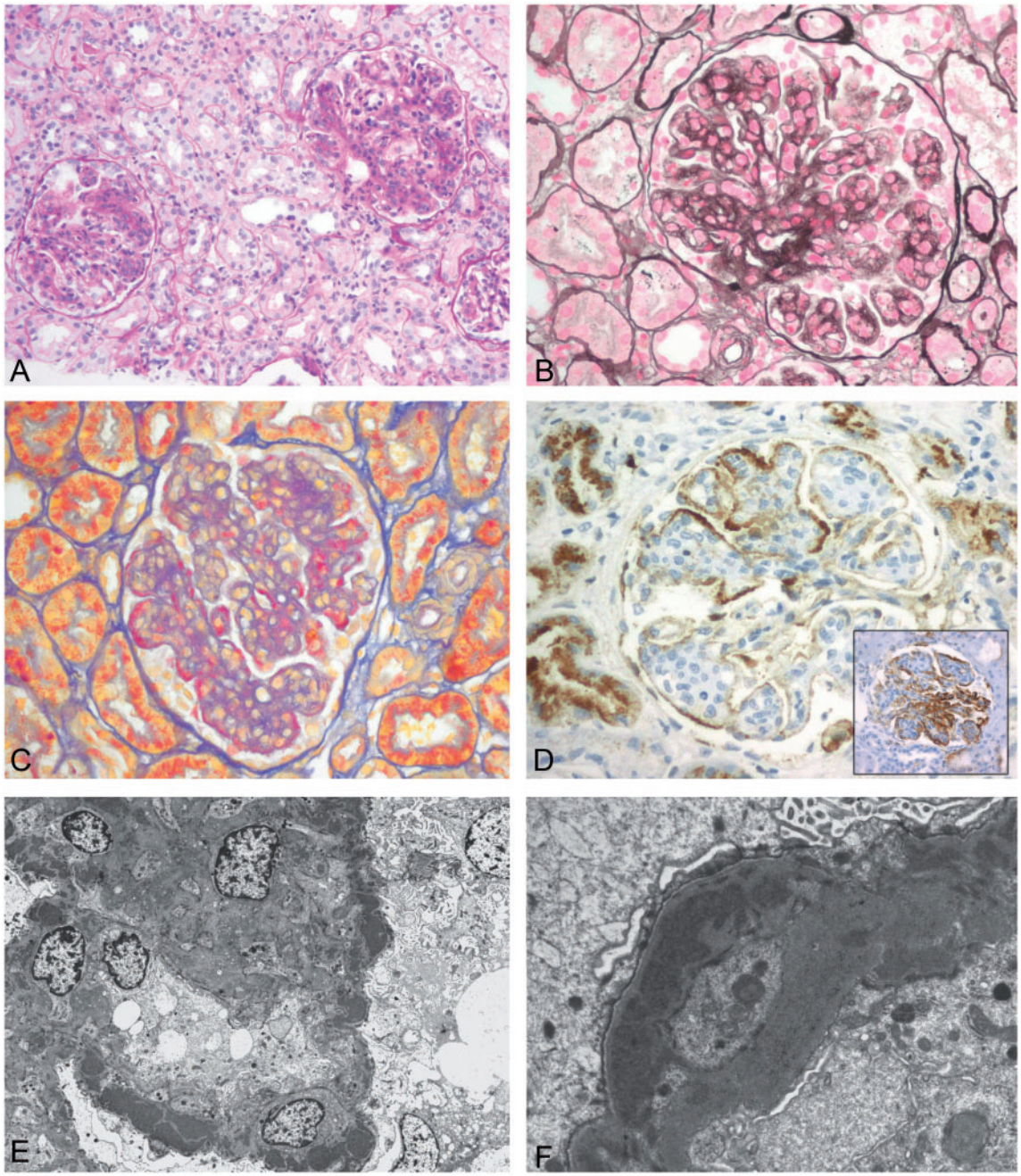

Fig. 3. MPGN I (Patient 1). (**A**) Mesangial and endocapillary proliferation (periodic acid–Schiff stain, original magnification ×110). (**B**) Splitting of the GBM (silver methenamine stain, original magnification ×220). (**C**) Subendothelial deposits (AFOG stain, original magnification ×200). (**D**) Immunhistochemistry positive for IgG (original magnification ×280), inset: positivity for C3 (original magnification ×220). (**E**) EM with mostly subendothelial and some subepithelial and mesangial electron-dense deposits (original magnification ×1950). (**F**) Subendothelial and small subepithelial electron-dense deposits (original magnification ×10 500).

after transplantation Therefore treatment with CSA was switched to tacrolimus and PEX was started [1.5-fold plasma volume exchange with fresh frozen plasma (FFP)], but ESRD occurred 3.9 years after RTPL. A deceased-donor RTPL was then performed. Immunosuppression included tacrolimus, MMF, PDN and induction with thymeoglobulin. Again, ABMR and recurrence of the disease with nephrotic proteinuria occurred 15 months after retransplantation. Weekly treatment with PEX reduced proteinuria, but proteinuria increased when the PEX interval was extended to every second week. Measurement of sC5b-9 (after PEX session) showed normal values. Renal graft function was impaired (eGFR 42 mL/min/1.73 m^2) at the last follow-up.

Discussion

MPGN and C3G are rare diseases with chronic progressive glomerulonephritis and children may have an unfavourable course leading to ESRD [17, 18, 44, 45]. Studies in adults and children have shown that proteinuria is a major risk factor for developing ESRD [1, 46, 47]. In the last decade, new insights are

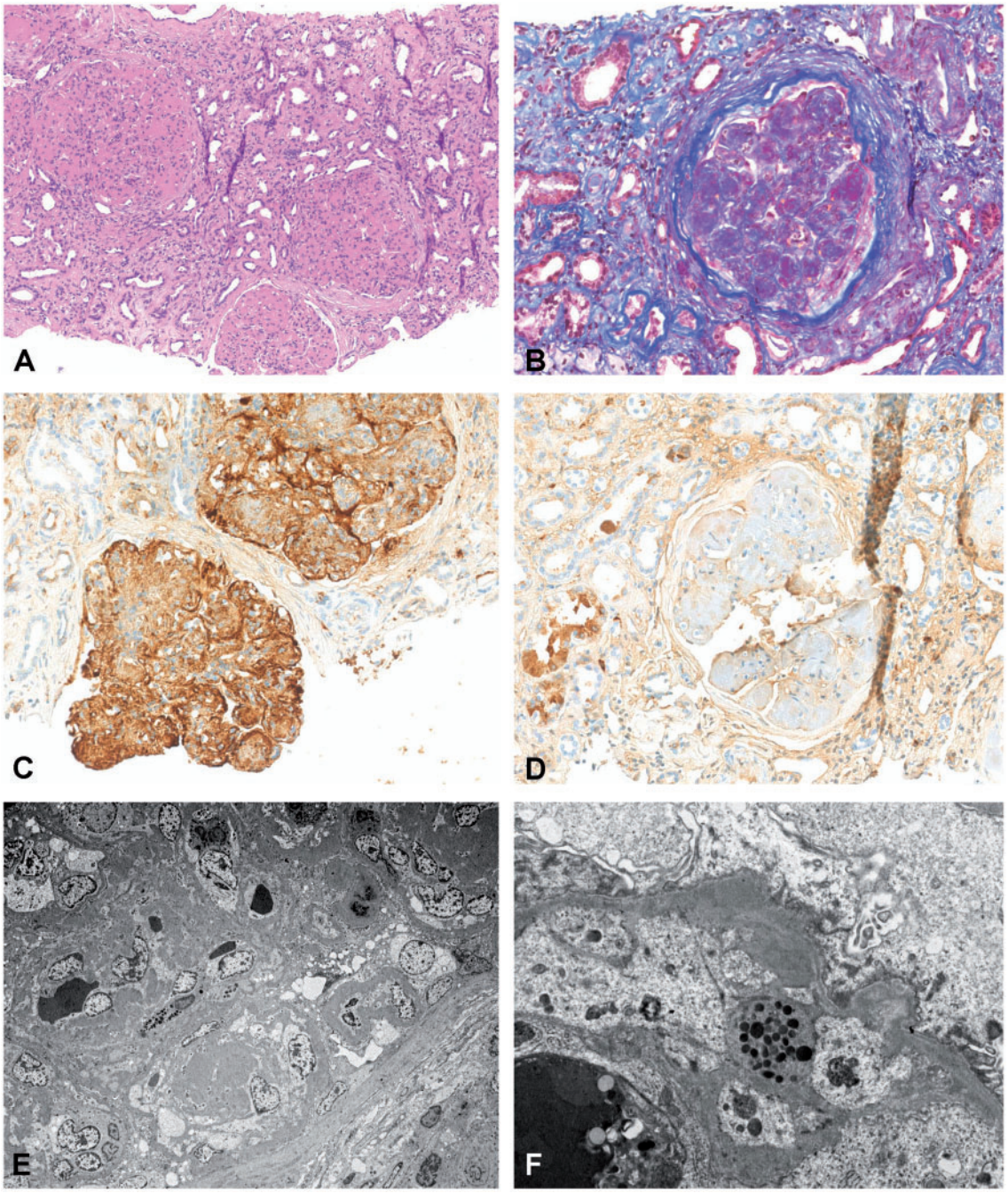

Fig. 4. C3GN (Patient 6). (**A**) Mesangial and endocapillary proliferation (H&E stain, original magnification ×100). (**B**) Mesangial and GBM deposits (AFOG stain, original magnification ×200). (**C**) Immunohistochemistry positive for C3 (original magnification ×200). (**D**) Immunohistochemistry negative for IgG (original magnification ×200). (**E**) EM with mostly intramembranous and mesangial not very dense electron-dense deposits (original magnification ×300). (**F**) EM with subepithelial, intramembranous and subendothelial deposits (original magnification ×2000).

emerging to improve genetic and biochemical investigations of these diseases.

Currently there is no established treatment for MPGN and C3G. Patients appear to respond differently to various therapy modalities [27, 29, 48, 49]. The majority of treatment regimens and case series have been reported in adults, not in children [23, 26], and are often associated with significant side effects. Recently, treatment with eculizumab, a monoclonal antibody binding to C5 of the alternative pathway, has shown promising results in the treatment of some cases of MPGN and C3G [22, 35–37, 50].

Our analysis revealed a dysregulation of the complement alternative pathway and mutation/variation in genes of CF proteins in children with MPGN, C3GN and DDD. Three patients of our series showed a favourable outcome. These were two patients with MPGN I and one with C3GN, the latter without any genetic variation in CFHR proteins. Five of seven children had a

Table 3. Patients with RAAS blockers only: values at last follow-up

Characteristic	Patient 1	Patient 2	Patient 3
Maintained renal function	Native kidney	Native kidney	Native kidney
Treatment	Enalapril	Enalapril Losartan	Enalapril Losartan
Duration of observation (years)	9.7	2.2	4.8
eGFR	143	97	180
C3 (g/L) (reference 0.7–1.76)	0.6	<0.06	0.13
UPCR (g/mol) (reference < 20)	41	41	<20
sC3d (mU/L) (reference < 40)	50	103	35
sC5b-9 (ng/mL) (reference < 320)	239	2538	164

eGFR rate according to Schwartz formula in mL/min/1.73 m^2.

Table 4. Patients with eculizumab: values before eculizumab and at last follow-up

Characteristic (original disease)	Patient 4 (MPGN I)	Patient 5 (C3GN)
Maintained renal function	Native kidney	Native kidney
Additional treatments to RAAS blockers	PDN MMF	
Before eculizumab		
Duration of observation (years)	6.9	9.5
eGFR (mL/min/1.73 m^2)	40	100
C3 (g/L) (reference 0.7–1.76)	0.09	0.06
UPCR (g/mol) (reference <20)	510	750
sC3d (mU/L) (reference <40)	63	95
sC5b-9 (ng/mL) (reference >320)	4100	6500
Observation on eculizumab		
Duration of therapy (months)	6	11
eGFR	45	90
UPCR (g/mol)	270	227
sC5b-9 (ng/mL)	282	639
C3 (g/L)	0.12	0.14

heterozygous mutation/deletion or variation in CFHR proteins 1, 2, 3 or 5. Recent findings show that dysregulation of the complement pathway by CFHR2–CFHR5 hybrid protein leads to enhanced C3-convertase activation of the alternative complement pathway and other genetic complement abnormalities associated with MPGN [12, 17, 51]. In our patients, genetic alterations, including variation or polymorphisms, may at least in part explain the different outcomes and responses to the various treatment modalities. Similar findings are described by other authors, where the clinical presentation and the measurement of plasma C3, C3d and sC5b-9 do not allow differentiation between C3G and MPGN I [22]. Recent knowledge suggests that in idiopathic MPGN—an immune complex Rixensart, Belgium mediated disease—involvement of the alternative pathway plays an important role [17, 52].

Three of seven of our patients (two with MPGN and one with C3GN) without nephrotic syndrome showed remission of proteinuria on RAAS blocker therapy only. No significant reduction in proteinuria is described in children with MPGN treated with RAAS blocker only. Other data revealed that patients with less parenchymal damage in their initial renal biopsy benefit the most from sole treatment with RAAS blocker [1]; however, spontaneous remission may not be excluded [22]. Consistent with other authors [1], those children of our series with nephrotic syndrome at onset showed a more severe course, with two progressing to ESRD followed by RTPL.

Eculizumab has been described as a successful treatment of patients with MPGN [35, 50, 53–55] and C3G [19, 22, 56] in several reports. Elevated sCb5-9 levels may be a predictor of response to treatment with eculizumab, but other factors affecting response to therapy are poorly understood [53, 55]. Two patients of our series, one with C3GN and one with MPGN I, exhibited a significant decrease in proteinuria on eculizumab in their native kidneys, but elevated activity of the alternative pathway persisted. This is consistent with observations of other paediatric patients with MPGN [50] suggesting that eculizumab is not completely effective in suppressing sC5b-9 activity in C3G. They hypothesized that sC5b-9 alone may not reflect disease activity. The presence of mutations alone does not significantly increase the risk of developing idiopathic MPGN or C3G, but they do so when combined with common susceptibility variants [i.e. in CD46, CFH or Thrombomodulin (THBD)] [17, 52].

C3NeF, an auto-antibody directed against the alternative C3-convertase, was positive at follow-up in only one child of our series. Published reports on the impact of C3NeF on outcome in C3G patients are inconsistent, with reported patients ranging from complete remission to ESRD [57, 58]. Apparently C3NeF can fluctuate during the clinical course independent of the disease treatment [22]. Others observed a higher risk of progressing to ESRD in patients without complement gene mutation or C3NeF, stabilizing the alternative pathway C3-convertase [52]. In our patient, C3NeF and a heterozygous variation of CFHR1 and CFHR1/3 was found. In addition, sC5b-9 was significantly elevated. Other treatments, for example, rituximab and PEX, are controversially discussed in the literature with different results [52, 58, 59]. Therefore, after a risk–benefit evaluation of these treatment options, we decided to apply eculizumab in our young child. The outcome was favourable, with stable, normal renal function and a significant decrease in proteinuria 11 months after eculizumab treatment was started.

In a patient undergoing RTPL twice, C3NeF and sC5b-9 were analysed after PEX treatment with FFP had been started, so detection of both C3NeF and sC5b-9 might have been missed [58]. This patient experienced aABMR with recurrence of DDD 3 years after the first RTPL and was therefore treated with PEX. However, he reached ESRD after the first RTPL, with recurrence of the disease in the second renal graft. Treatment with PEX was started at weekly intervals but led to only a moderate decrease in proteinuria with persistently impaired graft function. The insurance refused to pay for treatment with

Table 5. Patients undergoing RTPL: values before RTPL and at last follow-up after RTPL

Characteristic (original disease)	Patient 6 (C3GN)	Patient 7 (DDD)
Before RTPL		
Additional treatments to RAAS blockers	CSA	CSA
Duration of observation (years)	9	11.5
C3 (g/L) (reference 0.7–1.76)	0.2	0.55
UPCR (g/mol) (reference <20)	1350	400
sC3d (mU/L) (reference <40)	27	61
sC5b-9 (ng/mL) (reference <320)	1359	Not done
Observation after RTPL (m)		
Duration (months)	7	48 (2nd RTPL)
Recurrence of original disease (m)	No	Yes (15)
Treatment for original disease recurrence	No	PEX/FFP
Graft rejection	No	Yes (ABMR)
eGFR	51	42
UPCR (g/mol)	<20	400
sC5b-9 (ng/mL)	770	121
C3 (g/L)	0.25	0.9
Renal graft biopsy after RTPL	No	Yes
Treatment after RTPL	Induction with basiliximab for RTPL Prednisolone Tacrolimus MMF	Induction with thymeoglobulin for RTPL Prednisolone Tacrolimus MMF

eculizumab. As complement-mediated dysregulation is involved in the pathophysiology of aABMR [60], it is conceivable that DDD recurrence may have triggered aABMR by inducing complement activation. In a recent study, MPGN recurrence after RTPL was detected in 18 of 40 transplants and in 3 cases disease recurrence preceded aABMR and led to graft loss [4]. Registries have also reported recurrence rates on renal graft for DDD and C3G of 50% and 43–67%, respectively [22].

There are some limitations to our study: (i) its retrospective character; (ii) the individual treatment approach based on the clinical course; (iii) due to the different time period at disease manifestation of each patient, no uniformity of treatment was possible and (iv) measurement of activity of the alternative pathway (i.e. C3d, sC5b-9) and C3NeF was not available at disease onset.

Our results reveal that early examination of the alternative complement pathway may aid to define a more individually tailored treatment. However, we observed heterogeneity of clinical and biological features in MPGN and C3G and therefore the difficulty of interpretation of both genetic abnormalities and biochemical analysis. Long-term observation is necessary in order to draw conclusions about the results of treatments and renal function at follow-up.

Conflict of interest statement

None declared.

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